IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Deepak Rao
Group Art Unit	:	1624
Applicants	:	Guy W. Bemis et al.
Serial No.	:	09/336,266
Filed	:	June 18, 1999
For	:	INHIBITORS OF P38

New York, New York
March 8, 2001

Hon. Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF FRANCESCO GERALD SALITURO
UNDER 37 C.F.R. § 1.132

I, FRANCESCO GERALD SALITURO, a citizen of the United States of America, residing at 25 Baker Drive, Marlborough, MA (US), hereby declare that:

1. I am one of the named inventors of the above-identified patent application.

2. I received a B.S. in Life Science from the University of Wisconsin-Parkside, Kenosha, WI in 1980. In 1984, I received a Ph.D. in Pharmacy from the University of Wisconsin-Madison, Madison, WI. After receiving my Ph.D, I was a Post-Doctoral Research Associate at the University of Illinois in Champaign-Urbana from 1984-1986. From 1986-

1993, I was employed as a research scientist at Marion Merrell Dow Pharmaceuticals. A copy of my curriculum vitae is attached as Exhibit 1.

3. Since joining Vertex Pharmaceuticals, Inc. (hereafter "Vertex") in 1993, my work has been devoted to the design and evaluation of enzyme inhibitors as therapeutic treatments for human diseases. Over the last 4 years, I have worked on the design and evaluation of inhibitors of mitogen-activated protein kinases (MAPK), especially p38 protein kinase. I have co-authored two papers and have been a co-inventor on two issued patents and on six patent applications on MAP kinases.

4. I am familiar with the September 11, 2000 Office Action in the above-identified application. I understand that, in the Examiner's view, the specification does not teach how to make or use compounds of formulae (Ie), (If), (Ig) and (Ih) wherein Q_1 is other than phenyl or pyridyl, Q_2 is other than phenyl, thienyl, benzofuran, benzothiophene or indolyl and Q_3 is other than phenyl. See the September 11, 2000 Office Action, page 3, No. 1. Specifically, the Examiner states that A[t]he specification provides no sufficient enabling disclosure by way of representative examples or reasonable disclosure of starting material sources for the plethora of functional groups permitted at all Q1, Q2 and Q3 variables which include various cyclic moieties both carbocyclic and heterocyclic. See p. 3, No. 1.

I make this declaration for the following purposes:

a) to provide examples of p38 inhibitory compounds that fall within the claimed scope of the invention and that have been synthesized according to the teachings set forth in the specification; and

b) to demonstrate that the specification teaches one of ordinary skill in the art how to make the full scope of compounds that fall within the claimed scope of formulae (Ie), (If), (Ig) and (Ih) by following the teachings set forth in the application.

5. I have attached hereto Tables 1-3 showing compounds 501-544, 601 and 701-737. See Exhibit A.

6. The compounds were synthesized under my direct supervision or under my direction as part of a research collaboration. The above compounds were synthesized and purified according to the protocols disclosed in United States Patent Application 09/336,266. Specifically, the compounds were synthesized and purified according to synthetic schemes 7 and 8. See, page 37 of the specification. The compounds shown in Tables 1-3 in Exhibit A were characterized using magnetic resonance (NMR) spectroscopy. See, e.g., Example 1-4, pages 63-70 of the specification.

7. The p38 inhibitory activity of the compounds shown in Tables 1-3 in Exhibit A was tested in in vitro enzyme inhibition and in vitro human cell assays. The enzyme inhibition and cell assays that were used are described in

Example 17 and 18C of the patent application. See. e.g., page 94 and pages 97 to 98.

8. Compounds 501-544, 601 and 701-737 fall within the genus of formulae (Ig), (If) and (Ih) and further exemplify the claimed invention. Table 1 exemplifies compounds of formula Ig wherein radical Q3 is a substituted phenyl ring (see, e.g., compounds of formula 503, 507-518, 522-527 and 535-545), a substituted benzo[1,3]dioxole ring (see, e.g., compounds 501, 502, 505 and 506) or a substituted pyridyl ring (see, e.g., compounds 519-521, 528-534 and 544). The Ki and IC50 data demonstrate that the above compounds have p38 inhibitory activity. Thus, Table 1 demonstrates that additional compounds that fall within formula (Ig), including those in which Q1 is benzo[1,3]dioxole, have been synthesized and have p38 inhibitory activity.

Table 2 exemplifies a compound of formula (If), compound 601, wherein Q1 is a substituted phenyl and Q2 is phenyl. Table 2 shows that compound 601 has p38 inhibitory activity. See, e.g. the Ki and IC50 data for compound 601. Further, Table 3 shows compounds encompassed by formula (Ih), wherein Q1 is a substituted phenyl (see, e.g., compounds 701-712 and 714-737) or benzo[1,3]dioxole (compound 713), and wherein Q2 is substituted or unsubstituted phenyl. Table 3 demonstrates that these compounds have p38 inhibitory activity in vitro (see, e.g., the Ki data) and in vivo (see, e.g., the IC50 data). Table 1 also shows NMR spectroscopy and mass spectrometry data of the synthesized compounds.

9. I understand that claim 38 recites "Q3 is a 5-6 membered aromatic carbocyclic or heterocyclic ring system, or an 8-10 membered bicyclic ring system comprising aromatic carbocyclic rings, aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring" and "each of Q1 and Q2 are independently selected from 5-6 membered aromatic carbocyclic or heterocyclic ring systems, or 8-10 membered bicyclic ring systems consisting of aromatic carbocyclic rings consisting of aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring; wherein each said heterocyclic ring has a single heteroatom independently selected from N, O or S;". The original disclosure teaches the synthesis of the full scope of the compounds falling within the scope of formulae (Ie), (If), (Ig) and (Ih) as recited in claim 38 for the reasons presented in paragraph 9-11, infra.

10. The specification, as originally filed, teaches how to attach the Q1 ring system to the heterocyclic core to form compounds of formulae (If) and (Ih). See, e.g., schemes 7 and 8, step 1, pages 37-38. The disclosure teaches reacting a dibromopyridine derivative with an amine (Q1-NH2, e.g., aniline) in the presence of a base to make a bromopyridin phenyl amine derivative. Based on this disclosure, one having ordinary skill in the art would know how to synthesize analogous compounds of formulae (If) and (Ih) by using other amine-substituted aromatic carbocyclic or heterocyclic rings or amine-substituted bicyclic ring systems. These amine-substituted starting materials are commercially available or

are easy to synthesize by well-known methods. See, e.g., 4-aminopyridine (Fluka #36687), 5-aminoquinoline (Fluka #07340), 4-aminomorpholine (Aldrich #A6,630-8), 1-aminopiperidine (Aldrich #A7-590-0), 3-aminopyrazole (Aldrich #16,064-4), aminopyrazine (Aldrich #A7,695-8), 2-aminothiazole (Aldrich #12,312-9). Many other amine-substituted ring systems are commercially available or are readily synthesized. See, e.g., "Comprehensive Organic Transformations", Ed. R.C. Larock, Wiley-VCH Inc., pages 385-437 (1989) for general schemes for the synthesis of amines.

11. The originally filed specification also teaches several synthetic routes for attaching the Q2 ring system along with the spacer group X if present, to the heterocyclic core to form compounds of formulae (Ie), (Ig), (If) and (Ih). See, e.g., scheme 3, step 2, pages 26-27 and schemes 7 and 8, step 2, pages 37-38. The specification teaches a general route to synthesize compounds comprising the heterocyclic core by reacting a 2-amino-6-bromo-pyridine derivative with an aryl lithium compound. See, e.g., page 27, lines 2-4. Scheme 7 teaches an alternative route to synthesize compounds comprising the heterocyclic core by reacting a 2-amino-6-bromo-pyridine derivative with an arylstannane (Q2-Sn(R)3, e.g., tributylphenoltin) in the presence of a palladium catalyst. Scheme 8 teaches an another reaction protocol, using a phenylboronic acid (Q2-boronic acid, e.g., phenylboronic acid) in the presence of a palladium catalyst. Thus, based on this disclosure, one having ordinary skill in the art would know how to synthesize analogous compounds of formulae (Ie), (Ig), (If) and (Ih) by utilizing well-known

reactions to form compounds of the invention as described in the specification at pages 26-27. See, e.g., reactions of aryl lithium with heteroaryl compounds in "Advanced Organic Chemistry" Ed. Jerry March, page 666, John Wiley & Sons, Inc., forth Edition (1992). One may also follow the teachings in the specifications using other stannyl- or boronic acid-substituted aromatic carbocyclic or heterocyclic rings or amine-substituted bicyclic ring systems. See, e.g. pages 22-27 and schemes 7 and 8. Stannyl-substituted starting materials are commercially available. See, e.g., 2-(tributylstannyl)furan (Aldrich #41,450-6), 2-(tributyl-stannyl)thiophene (Aldrich #41,449-2), 2-tributyl-stannyl pyrazine (Frontier Scientific Fine Chemicals #T4192), 4-tributylstannylpyridine (Frontier Scientific Fine Chemicals #T3843). Boronic acid-substituted starting materials are also commercially available. See, e.g., phenylboronic acid (Aldrich #P2,000-9), naphthalene-2-boronic acid, (Frontier Scientific Fine Chemicals #N6202), 5-methylfuran-2-boronic acid (Frontier Scientific Fine Chemicals #M5933). Many other compounds that may be used as starting materials are commercially available or are readily synthesized.

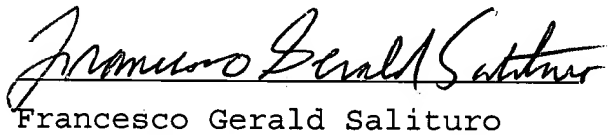
12. Further, the originally filed disclosure teaches how to attach the Q3 ring system to the heterocyclic core to form compounds of formulae (Ie) and (Ig). See, e.g., schemes 1-4, step 1, pages 24-27 and page 37, lines 2-4. The disclosure also teaches the reaction of a dichloropyridine derivative with a nitrile (Q3-CN, e.g., phenylacetonitrile) in the presence of a base to make a phenylpyridin acetonitrile derivative. See, e.g. scheme 1 for compounds

wherein Q1 is replaced by Q3, see also page 36, line 31 to page 37, line 2. Thus, based on the disclosure of the specification, one having ordinary skill in the art would know how to synthesize analogous compounds of formulae (Ie) and (Ig) by using other nitrile-substituted aromatic carbocyclic or heterocyclic rings or nitrile-substituted bicyclic ring systems. These nitrile-substituted starting materials are commercially available or easy to synthesize by well-known methods. See, 3-pyridyl-acetonitrile (Aldrich #P6,600-9), 2-naphthylacetonitrile (Aldrich #16,276-0), 2-thiophenylacetonitrile (Aldrich #14,168-2). For general synthesis of nitriles from carboxylic acids, see, e.g., "Comprehensive Organic Transformations", Ed. R.C. Larock, Wiley-VCH Inc., pages 976-977,(1989)). Thus, I believe that the specification showed how to attach a Q3 ring to synthesize compounds of formulae (Ie) and (Ig).

13. Therefore, for the reasons presented above in paragraph 5-11, I believe and reasonably expect that one of ordinary skill in the art would be able to synthesize compounds of formulae (Ie), (Ig), (If) and (Ih) with any of the claimed substituents for Q1, Q2 and Q3 following the teachings of the original disclosure.

14. I declare further that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section

1001, Title 18, United States Code, and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

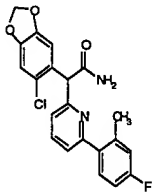
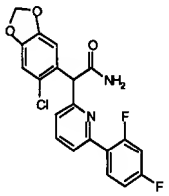
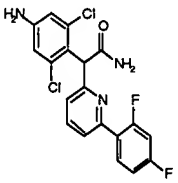
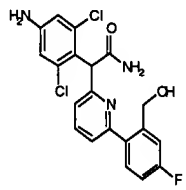
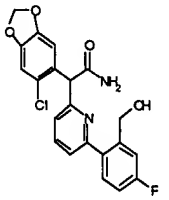
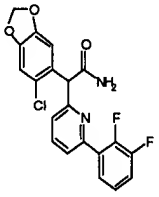
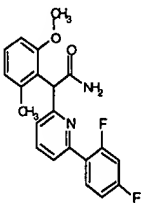


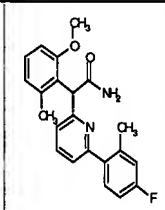
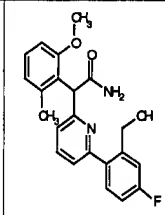
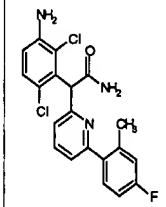
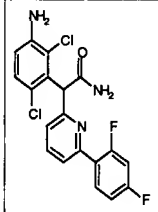
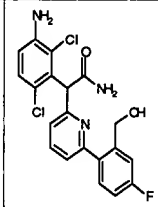
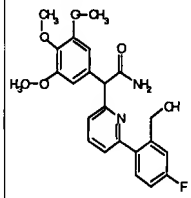
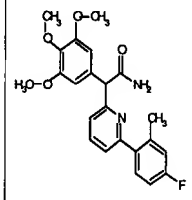
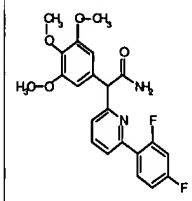
Francesco Gerald Salituro

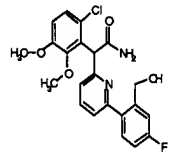
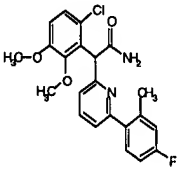
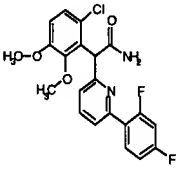
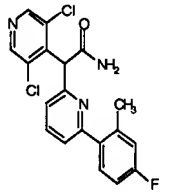
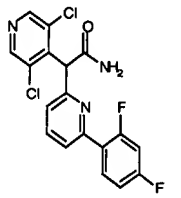
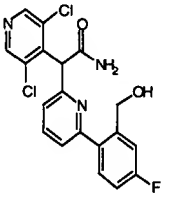
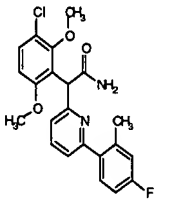
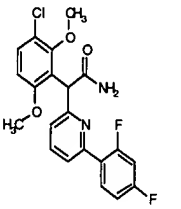
Signed this eighth day of March, 2001
at Cambdrige, USA.

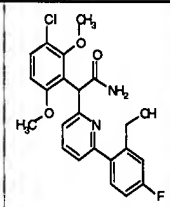
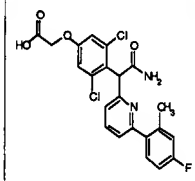
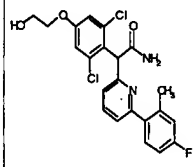
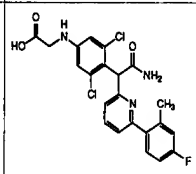
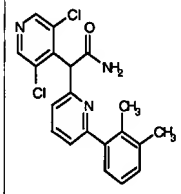
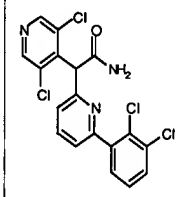
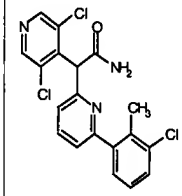
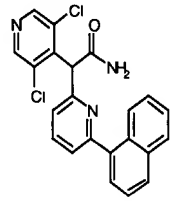
EXHIBIT A

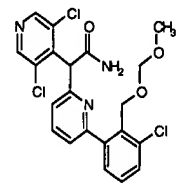
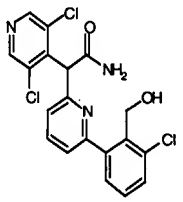
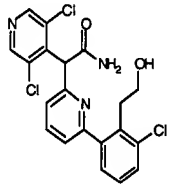
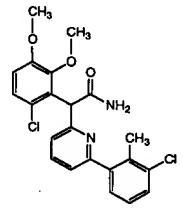
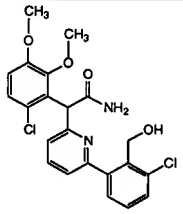
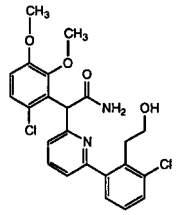
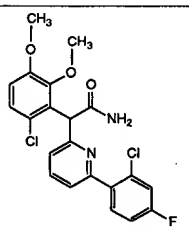
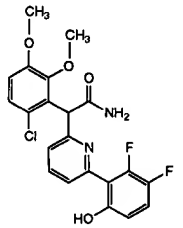
Table 1 (Ig)

Compound ID	Structure	Ki (μM)	Cell Assay IL1 (IC50)	Cell Assay TNF (IC50)	NMR or Mass Spec data	
501		2.88	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 7.72 (t, 1H), 7.38 (dd, 1H), 7.31 - 7.21 (dd, 2H), 7.11 (s, 1H), 7.01-6.95 (m, 2H), 6.89 (s, 1H), 5.97 (s, 2H), 5.69 (br s, 1H), 5.5 (s, 1H), 2.32 (s, 3H).	
502		6.17	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 8.09 (s, 2H), 8.07 - 8.0 (m, 1H), 7.80 (t, 1H), 7.70 - 7.60 (m, 2H), 7.20 (d, 1H), 7.12 -7.07 (m, 3H), 6.91 (s, 1H), 6.01 (d, 2H).	
503		0.073	0.15	6.7	^{1}H NMR (500 MHz, $CDCl_3$) d 7.95 (dd, 1H), 7.85 (t, 1H), 7.71 (br s, 1H), 7.80 - 7.51 (m, 2H), 7.40 (dd, 1H), 7.32 -7.20 (m, 2H), 7.17 (d, 1H), 6.64 (s, 2H), 5.79 (s, 2H).	
504		0.112	0.1	1.2	^{1}H NMR (500 MHz, $CDCl_3$) d 7.81 (t, 1H), 7.62 (br s, 1H), 7.50 (dd, 1H), 7.43 (d, 1H), 7.39 (dd, 1H), 7.23 (br s, 1H), 7.2 - 7.12 (m, 2H), 6.62 (s, 2H), 5.65 (s, 2H), 5.5 (s,1H), 5.21 (t, 1H), 4.5 (dd, 2H).	
505		10	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 7.80 (t, 1H), 7.52 - 7.45 (m, 2H), 7.30 (d, 1H), 7.20 (d, 1H), 7.12 - 7.07 (m, 1H), 7.02 (s, 1H), 6.89 (s, 1H), 6.12 (br s, 1H), 5.99 (s, 2H), 5.72 (br s, 1H), 5.51 (s, 1H), 5.39 (s, 1H), 4.45 (dd, 2H).	
506		10	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 7.79 (t, 1H), 7.71 - 7.6 (m, 2H), 7.30 (d, 2H), 7.29 - 7.19 (m, 2H), 7.13 (s, 1H), 6.89 (s, 1H), 5.95 (s, 2H), 5.59 (s, 1H).	
507		0.327	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 7.95 (dd, 1H), 7.89 (br s, 1H), 7.65 - 7.56 (m, 2H), 7.1 (t, 1H), 7.02 - 6.99 (m, 2H), 6.92 (t, 1H), 6.89 (d, 1H), 6.81 (d, 1H), 5.60 (br s, 1H), 5.58 (s, 1H), 3.75 (s, 3H), 2.33 (s, 3H).	

508		0.288	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 8.11 (br s, 1H), 7.60 (t, 1H), 7.39 (dd, 1H), 7.21 (t, 1H), 7.1 - 6.92 (m, 2H), 6.89 - 6.8 (dd, 2H), 6.89 (d, 1H), 6.77 (br s, 1H), 5.53 (s, 1H), 3.75 (s, 3H), 2.38 (s, 3H), 2.31 (s, 3H).	
509		1.465	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 7.79 (br s, 1H), 7.48 (dd, 1H), 7.41 (dd, 1H), 7.21 (t, 1H), 7.14 (t, 1H), 6.99 (d, 1H), 6.91 (d, 1H), 6.02 (br s, 1H), 6.51 (s, 1H), 5.50 (br s, 1H), 5.12 (br s, 1H), 4.40 (dd, 2H), 3.70 (s, 3H), 2.36 (s, 3H).	
510		0.12	1	6.7	^{1}H NMR (500 MHz, d6-DMSO) d 7.98 (br s, 1H), 7.80 (t, 1H), 7.45 - 7.36 (m, 2H), 7.31 (br s, 1H), 7.18 - 7.07 (m, 3H), 6.79 (d, 1H), 5.68 (s, 1H), 5.47 (s, 2H), 2.21 (s, 3H)	
511		0.128	N.D.	N.D.	^{1}H NMR (500 MHz, d6-DMSO) d 7.95 - 7.88 (m, 2H), 7.85 (t, 1H), 7.66 (d, 1H), 7.43 - 7.34 (m, 2H), 7.22 (t, 1H), 7.14 (dd, 2H), 6.80 (d, 1H), 5.68 (s, 1H), 5.50 (br s, 2H)	
512		0.294	N.D.	N.D.	^{1}H NMR (500 MHz, d6-DMSO) d 7.81 (t, 2H), 7.52 - 7.41 (m, 2H), 7.40 (dd, 1H), 7.30 (br s, 1H), 7.19 (t, 1H), 7.12 (d, 2H), 6.79 (d, 1H), 5.68 (s, 1H), 5.49 (br s, 2H), 5.20 (t, 1H), 4.5 (t, 2H).	
513		6.92	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 7.81 (t, 1H), 7.51 (dd, 1H), 7.49 (d, 1H), 7.41 (d, 1H), 7.22 - 7.20 (m, 1H), 7.10 (t, 1H), 6.70 (s, 2H), 6.10 (br s, 1H), 5.62 (br s, 1H), 5.55 (br s, 1H), 5.05 (s, 1H), 4.49 (dd, 2H), 3.82 (s, 9H).	
514		4.33	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 7.75 (t, 2H), 7.37 (dd, 1H), 7.30 (dd, 1H), 7.03 - 6.95 (m, 2H), 6.52 (br s, 1H), 4.99 (t, 1H), 3.80 (s, 9H), 2.25 (s, 3H).	
515		4.38	0.4	0.81	^{1}H NMR (500 MHz, $CDCl_3$) d 7.95 (dd, 1H), 7.79 (t, 1H), 7.69 (br s, 1H), 7.65 (d, 1H), 7.28 (s, 2H), 7.01 (t, 1H), 6.95 (t, 1H), 6.74 (s, 1H), 5.55 (br s, 1H), 4.99 (s, 1H), 3.81 (s, 9H).	

516		0.362	6.7	6.7	^{1}H NMR (500 MHz, $CDCl_3$) d 7.79 (dd, 1H), 7.45 (t, 1H), 7.42 (d, 1H), 7.2 - 7.12 (m, 2H), 7.08 (dt, 1H), 6.88 (d, 1H), 6.45 (br s, 1H), 5.77 (s, 1H), 5.51 (br s, 1H), 4.71 (t, 1H), 4.41 (d, 2H), 3.85 (s, 3H), 3.66 (s, 3H).	
517		0.131	2.4	6.7	^{1}H NMR (500 MHz, $CDCl_3$) d 9.11 (br s, 1H), 7.61 (t, 1H), 7.39 (dd, 1H), 7.20 (d, 1H), 7.17 (m, 1H), 7.02 - 6.96 (m, 2H), 6.92 - 6.87 (m, 2H), 6.70 (s, 2H), 3.89 (s, 3H), 3.79 (s, 3H), 2.39 (s, 3H).	
518		0.164	6.7	6.7	^{1}H NMR (500 MHz, $CDCl_3$) d 8.75 (br s, 1H), 7.91 (dd, 1H), 7.68 - 7.58 (m, 2H), 7.18 (d, 1H), 7.02 (dt, 1H), 6.97 - 6.90 (m, 2H), 6.89 (d, 1H), 5.71 (s, 2H), 4.89 (s, 3H), 4.78 (s, 3H).	
519		0.297	5.3	6.7	^{1}H NMR (500 MHz, $CDCl_3$) d 9.62 (br s, 1H), 8.58 (s, 2H), 7.70 (t, 1H), 7.40 (dd, 1H), 7.32 (d, 1H), 7.07 - 6.97 (m, 2H), 6.74 (d, 1H), 5.90 (br s, 1H), 5.89 (s, 1H), 2.29 (s, 3H).	
520		0.69	6.7	6.7	^{1}H NMR (500 MHz, d6-DMSO) d 8.61 (s, 2H), 7.90 (t, 1H), 7.70 (t, 1H), 7.81 - 7.71 (m, 1H), 7.70 (d, 1H), 7.52 (br s, 1H), 7.41 - 7.31 (m, 2H), 7.20 (dt, 1H), 5.80 (s, 1H).	
521		1.04	N.D.	N.D.	^{1}H NMR (500 MHz, d6-DMSO) d 8.60 (s, 2H), 7.90 (t, 1H), 7.79 (br s, 1H), 7.51 (br s, 1H), 7.50 (d, 1H), 7.45 - 7.37 (m, 2H), 7.32 (d, 1H), 7.13 (dt, 1H), 5.80 (s, 1H), 5.11 (t, 1H), 4.30 (m, 2H).	
522		0.697	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 8.73 (br s, 1H), 7.61 (t, 1H), 7.39 (t, 1H), 7.32 (d, 1H), 7.21 (d, 1H), 7.02 - 6.94 (m, 3H), 6.69 (d, 1H), 5.61 (br s, 1H), 5.60 (s, 1H), 3.85 (s, 3H), 3.71 (s, 3H), 2.39 (s, 3H).	
523		0.594	6.7	6.7	^{1}H NMR (500 MHz, $CDCl_3$) d 8.39 (br s, 1H), 7.91 (m, 1H), 7.66 - 7.58 (m, 2H), 7.32 (d, 1H), 7.01 (dt, 1H), 6.98 (d, 1H), 6.92 (t, 1H), 6.70 (d, 1H), 5.69 (br s, 1H), 5.61 (s, 1H), 3.83 (s, 3H), 3.72 (s, 3H).	

524		5.31	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 7.79 (t, 1H), 7.50 - 7.40 (m, 3H), 7.31 (d, 1H), 7.19 (d, 1H), 7.09 (t, 1H), 6.70 (d, 1H), 6.30 (br s, 1H), 5.70 (s, 1H), 5.55 (br s, 1H), 4.99 (br s, 1H), 4.40 (q, 2H), 3.80 (d, 3H), 3.75 (s, 3H).	
525		0.129	2.2	2.2	^{1}H NMR (500 MHz, d6-DMSO) d 7.95 - 7.82 (m, 2H), 7.50 - 7.35 (m, 2H), 7.21 (d, 1H), 7.20 - 7.08 (m, 2H), 5.70 (s, 1H), 4.80 (s, 2H), 2.10 (s, 3H).	
526		0.44	2.2	2.2	^{1}H NMR (500 MHz, $CDCl_3$) d 9.80 (br s, 1H), 7.69 (t, 1H), 7.40 (dd, 1H), 7.30 (d, 1H), 7.02 (m, 3H), 6.80 (d, 1H), 5.80 (s, 2H), 4.10 (br s, 2H), 3.98 (br s, 2H), 2.40 (s, 3H).	
527		0.129	2.2	2.2	^{1}H NMR (500 MHz, d6-DMSO) d 8.01 (br s, 1H), 7.80 (br s, 1H), 7.57 (m, 1H), 7.49 (dd, 1H), 7.40 (br s, 1H), 7.27 - 7.12 (m, 2H), 6.70 (s, 1H), 5.61 (s, 1H), 3.89 (s, 2H), 2.29 (s, 3H).	
528		0.091	0.071	0.271	^{1}H NMR (500 MHz, $CDCl_3$) d 9.92 (br s, 1H), 8.59 (s, 2H), 7.69 (t, 1H), 7.33 (d, 1H), 7.23 (d, 1H), 6.70 (d, 1H), 5.90 (s, 1H), 5.80 (br s, 1H), 3.98 (br s, 2H), 2.40 (s, 3H), 2.23 (s, 3H).	
529		0.077	0.136	0.443	^{1}H NMR (500 MHz, $CDCl_3$) d 9.50 (br s, 1H), 8.60 (s, 2H), 7.72 (t, 1H), 7.60 (d, 1H), 7.51 (d, 1H), 7.42 (d, 1H), 7.38 (t, 1H), 6.80 (d, 1H), 5.90 (s, 1H), 5.81 (br s, 1H).	
530		0.068	0.211	1.01	^{1}H NMR (500 MHz, $CDCl_3$) d 9.69 (br s, 1H), 8.59 (s, 2H), 7.71 (t, 1H), 7.49 (d, 1H), 7.32 (d, 1H), 7.35 - 7.20 (m, 1H), 6.79 (d, 1H), 5.90 (s, 2H), 5.87 (br s, 1H), 2.28 (s, 3H).	
531		0.215	2.2	2.2	^{1}H NMR (500 MHz, $CDCl_3$) d 9.90 (br s, 1H), 8.61 (s, 2H), 8.10 (d, 1H), 8.0 (t, 2H), 7.79 (t, 1H), 7.70 - 7.49 (m, 4H), 6.80 (d, 1H), 5.92 (s, 1H), 5.71 (br s, 1H).	

532		0.1	0.112	0.274	^{1}H NMR (500 MHz, $CDCl_3$) d 9.50 (br s, 1H), 8.60 (s, 2H), 7.71 (t, 1H), 7.60 - 7.50 (m, 2H), 7.40 (d, 1H), 6.80 (d, 1H), 5.89 (s, 1H), 5.79 (br s, 1H), 5.71 (br s, 1H), 4.69 (m, 4H), 3.33 (s, 3H).	
533		0.096	0.03	0.383	^{1}H NMR (500 MHz, $CDCl_3$) d 8.59 (s, 2H), 8.0 (br s, 1H), 7.80 (t, 1H), 7.51 (d, 2H), 7.40 (m, 2H), 7.10 (d, 1H), 5.91 (s, 1H), 5.80 (br s, 1H), 4.68 (m, 2H).	
534		0.147	0.039	0.16	^{1}H NMR (500 MHz, $CDCl_3$) d 8.59 (s, 2H), 7.91 (br s, 1H), 7.78 (t, 1H), 7.50 (d, 1H), 7.40 (d, 1H), 7.29 (d, 1H), 7.0 (d, 1H), 5.91 (s, 1H), 5.78 (br s, 1H), 3.82 (m, 2H), 3.09 (m, 2H), 3.0 (t, 1H).	
535		0.07	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 9.20 (br s, 1H), 7.62 (t, 1H), 7.43 (d, 1H), 7.30 (d, 1H), 7.15 (d, 1H), 6.91 (d, 1H), 6.89 (d, 1H), 5.71 (s, 1H), 5.69 (br s, 1H), 3.89 (s, 3H), 3.80 (s, 3H).	
536		0.13	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 7.79 (t, 1H), 7.49 (d, 1H), 7.41 (dd, 1H), 7.33 - 7.28 (m, 1H), 7.17 (d, 1H), 6.87 (d, 1H), 6.71 (br s, 1H), 5.78 (s, 1H), 5.51 (br s, 1H), 5.30 (s, 1H), 4.61 (d, 2H), 4.12 (t, 1H), 3.86 (s, 3H), 3.68 (s, 3H).	
537		0.119	0.25	0.57	^{1}H NMR (500 MHz, $CDCl_3$) d 7.72 (dd, 1H), 7.47 (d, 1H), 7.31 (d, 1H), 7.33 - 7.28 (m, 1H), 7.18 (d, 1H), 6.88 (d, 1H), 6.3 (br s, 1H), 5.80 (s, 1H), 5.30 (s, 1H), 4.51 (t, 1H), 3.91 (m, 2H), 3.88 (s, 3H), 3.71 (s, 3H), 3.10 (m, 2H).	
538		0.145	0.31	6.7	^{1}H NMR (500 MHz, $CDCl_3$) d 9.01 (br s, 1H), 7.65 (t, 1H), 7.59 (dd, 1H), 7.48 (d, 1H), 7.18 (d, 2H), 7.11 (dt, 1H), 6.94 (d, 1H), 6.90 (d, 1H), 5.71 (s, 1H), 5.69 (br s, 1H), 3.90 (m, 3H), 3.79 (s, 3H).	
539		2.994	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 13.0 (s, 1H), 7.99 (d, 1H), 7.85 (t, 1H), 7.50 (d, 1H), 7.20 (d, 1H), 7.08 (q, 1H), 6.90 (d, 1H), 6.70 (dd, 1H), 6.02 (br s, 1H), 5.79 (s, 1H), 5.52 (br s, 1H), 4.89 (s, 3H), 3.62 (s, 3H).	

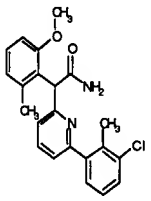
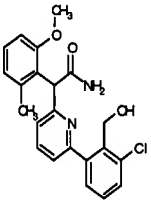
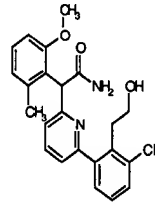
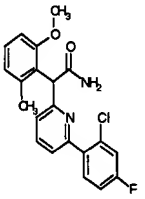
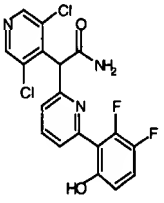
540		0.216	0.62	0.85	^{1}H NMR (500 MHz, $CDCl_3$) d 8.21 (br s, 1H), 7.61 (t, 1H), 7.42 (d, 1H), 7.29 (d, 1H), 7.21 (m, 2H), 7.02 (d, 1H), 6.93 (dd, 2H), 5.55 (s, 2H), 3.78 (s, 1H), 2.36 (s, 3H), 2.31 (s, 3H).	
541		0.469	0.44	1.9	^{1}H NMR (500 MHz, $CDCl_3$) d 7.78 (t, 1H), 7.49 - 7.4 (m, 3H), 7.34 (d, 1H), 7.30 (d, 1H), 7.21 (t, 1H), 6.86 (dd, 1H), 6.18 (br s, 1H), 5.51 (s, 1H), 4.60 (m, 3H), 3.70 (s, 3H), 2.28 (s, 3H).	
542		0.421	0.37	1.4	^{1}H NMR (500 MHz, $CDCl_3$) d 7.71 (t, 1H), 7.45 (d, 1H), 7.31 - 7.20 (m, 6H), 6.89 (dd, 2H), 5.90 (br s, 1H), 5.53 (s, 1H), 5.42 (br s, 1H), 4.91 (m, 2H), 3.78 (s, 3H), 3.07 (m, 2H), 2.28 (s, 3H).	
543		0.233	0.55	6.7	^{1}H NMR (500 MHz, $CDCl_3$) d 8.15 (br s, 1H), 7.61 (t, 1H), 7.58 (dd, 1H), 7.43 (d, 1H), 7.25 - 7.19 (m, 1H), 7.10 (t, 1H), 7.01 (d, 1H), 6.85 (dd, 2H), 5.55 (s, 2H), 3.78 (s, 3H), 2.31 (s, 3H).	
544		1.096	N.D.	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 12.42 (br s, 1H), 8.61 (br s, 1H), 8.02 (d, 1H), 7.92 (t, 1H), 7.49 (d, 1H), 7.10 (q, 1H), 6.70 (dd, 1H), 5.84 (br s, 2H), 5.55 (s, 2H).	
"N.D." represents value not determined						

Table 2 (If)

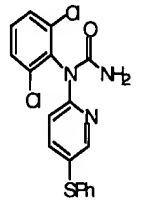
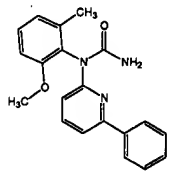
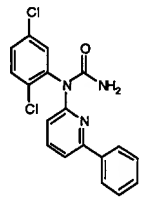
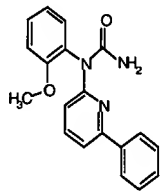
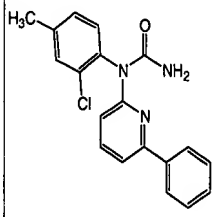
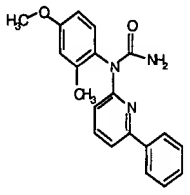
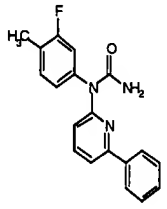
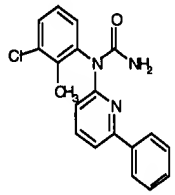
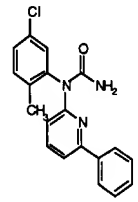
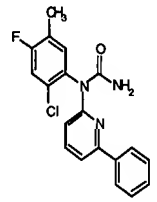
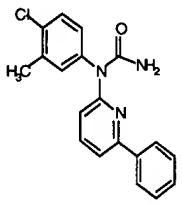
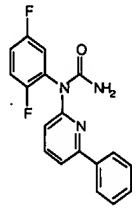
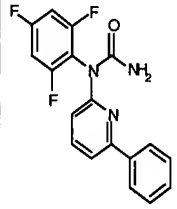
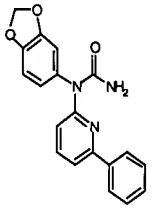
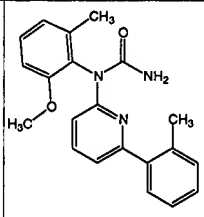
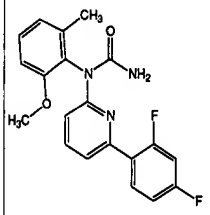
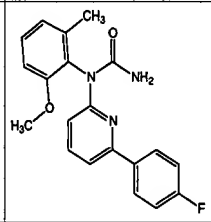
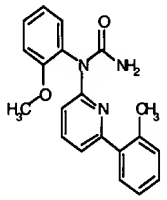
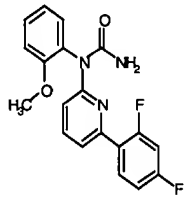
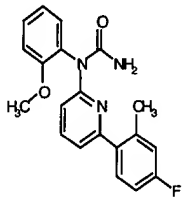
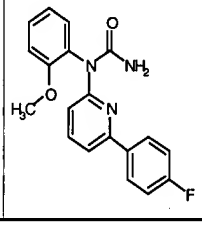
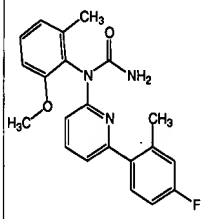
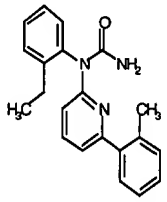
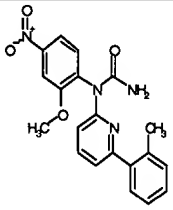
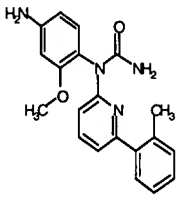
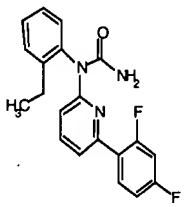
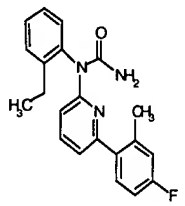
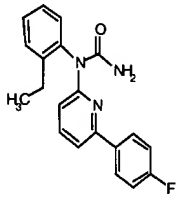
Compound ID	Structure	Ki (μM)	Cell Assay IL1 (IC50)	Cell Assay TNF (IC50)	NMR or Mass Spec data
601		0.9			^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.53 - 7.48 (m, 3H), 7.28 - 7.2 (m, 5H), 6.21 (s, 2H).

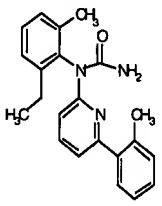
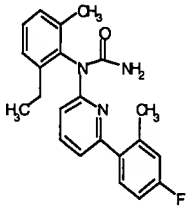
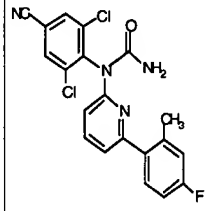
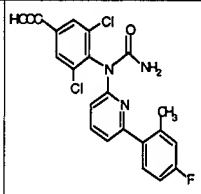
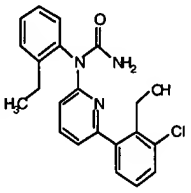
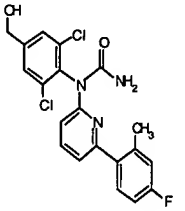
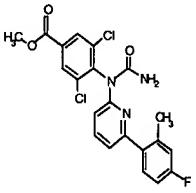
Table 3 (Ih)

Compound ID	Structure	Ki (μM)	Cell Assay IL1 (IC50)	Cell Assay TNF (IC50)	NMR or Mass Spec data
701		0.13	1.4	2.8	Expected Mass = 333.15; Actual Mass (M + H^+) = 334.1
702		0.44	1.8	2.9	Expected Mass = 357.04; Actual Mass (M + H^+) = 358.2
703		0.25	N.D.	N.D.	Expected Mass = 391.00; Actual Mass (M + H^+) = 392.1
704		0.165	0.56	1.2	Expected Mass = 337.10; Actual Mass (M + H^+) = 338.1
705		0.145	0.74	5	Expected Mass = 333.14; Actual Mass (M + H^+) = 334.2

Compound ID	Structure	Ki (μM)	Cell Assay IL1 (IC50)	Cell Assay TNF (IC50)	NMR or Mass Spec data
706		1.03	2.6	6.6	Expected Mass = 321.12; Actual Mass (M + H^+) = 322.2
707		0.14	1	4.3	Expected Mass = 337.1; Actual Mass (M + H^+) = 338.1
708		0.33	2	6.7	Expected Mass = 337.1; Actual Mass (M + H^+) = 338.1
709		0.73	1.8	6.7	Expected Mass = 355.09; Actual Mass (M + H^+) = 356.2
710		1.7	6.3	6.7	Expected Mass = 337.1; Actual Mass (M + H^+) = 338.1
711		0.8	0.58	6.7	Expected Mass = 325.10; Actual Mass (M + H^+) = 326.1
712		0.33	0.23	1	Expected Mass = 343.09; Actual Mass (M + H^+) = 344.1
713		N.D.	0.56	0.19	Expected Mass = 333.11; Actual Mass (M + H^+) = 334.1

Compound ID	Structure	Ki (μM)	Cell Assay IL1 (IC50)	Cell Assay TNF (IC50)	NMR or Mass Spec data
714		0.01	0.27	0.36	Expected Mass = 347.16; Actual Mass (M + H^+) = 348.2
715		0.023	0.16	0.1	Expected Mass = 369.12; Actual Mass (M + H^+) = 370.3
716		0.055	0.29	0.19	Expected Mass = 351.53; Actual Mass (M + H^+) = 352.3
717		0.07	0.35	0.14	Expected Mass = 333.15; Actual Mass (M + H^+) = 334.2
718		0.04	0.091	0.041	Expected Mass = 355.11; Actual Mass (M + H^+) = 356.3
719		0.045	0.48	0.13	Expected Mass = 351.14; Actual Mass (M + H^+) = 352.3
720		0.14	0.5	0.33	Expected Mass = 337.12; Actual Mass (M + H^+) = 338.1

Compound ID	Structure	Ki (μM)	Cell Assay IL1 (IC50)	Cell Assay TNF (IC50)	NMR or Mass Spec data
721		0.03	0.079	0.005	Expected Mass = 365.15; Actual Mass (M + H^+) = 366.3
722		0.01	0.15	0.055	Expected Mass = 331.16; Actual Mass (M + H^+) = 332.2
723		0.35	0.59	0.75	Expected Mass = 378.13; Actual Mass (M + H^+) = 379.3
724		0.25	0.23	0.3	Expected Mass = 348.16; Actual Mass (M + H^+) = 349.2
725		0.012	0.052	0.054	Expected Mass = 353.13; Actual Mass (M + H^+) = 354.3
726		0.015	0.071	0.069	Expected Mass = 349.16; Actual Mass (M + H^+) = 350.2
727		0.065	0.13	0.2	Expected Mass = 381.12; Actual Mass (M + H^+) = 382.2

Compound ID	Structure	Ki (μM)	Cell Assay IL1 (IC50)	Cell Assay TNF (IC50)	NMR or Mass Spec data
728		0.006	0.63	0.26	Expected Mass = 344.19; Actual Mass (M + H^+) = 346.2
729		N.D.	0.23	0.89	Expected Mass = 363.17; Actual Mass (M + H^+) = 364.3
730		0.115	0.39	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 8.80 (s, 1H), 7.64 (t, 1H), 7.40 (dd, 1H), 7.11 (d, 1H), 7.07 - 6.97 (m, 3H), 6.12 (d, 1H), 2.40 (s, 3H).
731		0.055	0.21	N.D.	^{1}H NMR (500 MHz, $CDCl_3$) d 7.90 (d, 2H), 7.61 (dd, 1H), 7.41 (dd, 1H), 7.10 (d, 1H), 7.03 (m, 2H), 6.83 (br s, 1H), 6.12 (d, 1H), 5.80 (br s, 1H), 2.41 (s, 3H).
732		0.007	0.049	0.38	Expected Mass = 424.1302; Actual Mass (M + H^+) = 425.3
733		3.5	6.7	6.7	^{1}H NMR (500 MHz, $CDCl_3$) d 7.51 (t, 1H), 7.41 (s, 2H), 6.95 (m, 2H), 6.83 (d, 1H), 6.58 (br s, 1H), 6.26 (d, 1H), 5.10 (s, 2H), 4.75 (br s, 2H), 2.40 (s, 3H).
734		0.006	0.024	2.15	^{1}H NMR (500 MHz, $CDCl_3$) d 8.17 (s, 1H), 7.62 (t, 1H), 7.41 (dd, 1H), 7.10 (d, 1H), 7.01 (m, 3H), 6.18 (d, 1H), 4.80 (br s, 1H), 3.99 (s, 3H), 2.40 (s, 3H).

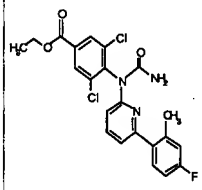
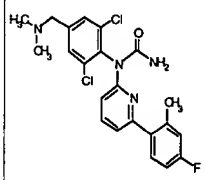
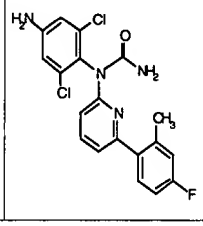
Compound ID	Structure	Ki (μM)	Cell Assay IL1 (IC50)	Cell Assay TNF (IC50)	NMR or Mass Spec data
735		0.011	0.018	0.245	^{1}H NMR (500 MHz, $CDCl_3$) d 8.18 (s, 1H), 7.60 (t, 1H), 7.41 (dd, 1H), 7.10 (d, 1H), 7.07 - 6.98 (m, 2H), 6.18 (d, 1H), 4.42 (q, 2H), 1.42 (s, 3H).
736		0.038	0.32	0.78	Expected Mass = 446.1; Actual Mass (M + H^+) = 448.1
737		1.79	5	6.7	Expected Mass = 404.46; Actual Mass (M + H^+) = 405.1
"N.D." represents value not determined					

Exhibit J

FRANCESCO G. SALITURO, Ph.D.

Home:

25 Baker Dr.
Marlborough, MA 01752
(508) 480-9610

Office:

Vertex Pharmaceuticals, Inc.
130 Waverly St.
Cambridge MA 02139

Education and Experience:

1997-Present **Senior Staff Investigator**
Vertex Pharmaceuticals
Continued as chemistry group leader in p38 Map Kinase project through 1998. Currently Project Leader for JNK Kinase program.

1993-1997 **Staff Scientist**
Vertex Pharmaceuticals
Primary Responsibilities from 1993-present:
Chemistry Group Leader for HIV Protease 2nd generation project (through sept.1996) and for p38 Map Kinase project. Supervised groups consisting of up to 7 members, both Ph.D. and non-Ph.D. and coordinated chemistry research collaborations with corporate partners on both programs.

1990 - 1993 **Sr. Associate Scientist**
Marion Merrell Dow Research Institute
Primary responsibilities from 1986-1993: Bench chemist supervising one Masters level chemist. Research focus on the design and synthesis of NMDA Glycine Site Antagonists.

1992 Department Safety Representative

1988 - 1991 Assistant Safety Representative
Department of Discovery Chemistry

1986 - 1989 **Sr. Research Chemist I**
Merrell Dow Research Institute

1984 - 1986 **UNIVERSITY OF ILLINOIS, Champaign-Urbana,**
Post-Doctoral Research Associate with Professor John A. Katzenellenbogen. Synthesis of [^{125}I]iodota-moxifen aziridine, a non-steroidal, anti-estrogenic affinity label and

radiolabel for the estrogen receptor. Synthesis of haloenol lactone inhibitors of elastase.

1980 - 1984 **UNIVERSITY OF WISCONSIN-Madison; Madison, Wisconsin.**
Ph.D. (Medicinal Chemistry); October, 1984.
Dissertation research, under the direction of Professor Daniel H. Rich, involved synthetic and kinetic studies of pepstatin analogs as potent, selective aspartyl protease inhibitors.

1982 - 1983 Served as substitute lecturer for an undergraduate Medicinal Chemistry course at the University of Wisconsin-Madison.

1976 - 1980 **UNIVERSITY OF WISCONSIN-Parkside; Kenosha, Wisconsin.**
B.S. (Life Science), Magna Cum Laude, 1980. Undergraduate research (1978-1980) in Bioorganic Chemistry under Professor Bruce R. Branchini. participated in a project aimed at the synthesis, development and kinetic analysis of chemiluminescent substrates for serine proteases.

Personal Data:
Born: February 15, 1958; Kenosha, Wisconsin.
Height: 5'8"; Weight: 175 lbs.; Health: Excellent.
Marital Status: Married; three children.

Interests and Hobbies: outdoor sports, fishing, music

Honors and Organizations:
Magna Cum Laude Graduate (UW-Parkside);
Certificate of Merit, Life Science Department, UW-Parkside;
American Chemical Society.

Journal Referee
Journal of Medicinal Chemistry
Bioorganic and Medicinal Chemistry Letters
Journal of Organic Chemistry

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23. "MDL 100,458 and MDL 102,288: Two potent and Selective Glycine Antgonists With Different Functional Profiles," J.H. Kehne, B.B. Baron, B.L. Harrison, T.C. McCluskey, M.G. Palfreyman, M. Poirot, **F.G. Salituro**, B.W. Siegel, A.L. Slone, P. van Giersbergen, H.S. White, Eur. J. Pharmacol., 284, 109-118 (1995)

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30. " Inhibitors of p38 MAP kinase: therapeutic intervention in cytokine-mediated diseases."**Salituro FG**, Germann UA, Wilson KP, Bemis GW, Fox T, Su MS. Curr Med Chem. 1999 Sep;6(9):807-23.

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32. "Structure, synthesis, and biological activity of VX-745: A novel, orally bioavailable and selective p38 MAP kinase inhibitor" **Francesco G. Salituro,** Guy W. Bemis, Ursula A.Germann, John P. Duffy,Vincent P. Galullo, Edmund M. Harrington, S. Pazhanisamy, Pamela J. Ford, Karyn L. Cepek, Yow-Ming C. Wang, Steven F. Bellon, George Ku, Keith P.Wilson, Michael S.-S Su. Manuscript submitted for publication

US Issued PATENTS

1.
6,093,742 Inhibitors of p38
2.
5,945,418 Inhibitors of p38
3.
5,945,413 Aspartyl protease inhibitors
4.
5,883,252 Aspartyl protease inhibitors
5.
5,877,202 Indole derivatives useful to treat estrogen-related neoplasms and disorders
6.
5,703,107 3-aminoindolyl derivatives
7.
5,675,018 3-amidoindolyl derivatives
8.
5,547,991 NMDA antagonism method
9.
5,519,048 3-(indol-3-yl)-propenoic acid derivatives and pharmaceutical compositions thereof
10.
5,491,153 3-amidoindolyl derivative
11.
5,484,814 NMDA antagonists
12.
5,470,870 NMDA antagonists
13.
5,360,814 NMDA antagonists
14.
5,189,054 3-amidoindolyl derivatives and pharmaceutical compositions thereof
15.
5,106,847 Excitatory amino acid antagonists, compositions and use
16.
5,051,442 3-indolyl thioacetate derivatives and NMDA receptor antagonistic use thereof
17.
4,960,786 Excitatory amino acid antagonists

Selected Presentations

1. "Design and Synthesis of Direct Acting and Enzyme Activated NMDA Glycine Site Antagonists" Invited speaker at the 7th RSC-SCI Medicinal Chemistry Symposium, Sept 1993.

2. "Synthesis and SAR of a Novel Series of HIV Aspartyl Protease Inhibitors" Poster Presentation at the 2nd Winter Conference on Medicinal Chemistry, Steamboat CO. , Jan 1997.

3. "Design and Synthesis of Novel Conformationally Restricted HIV Protease Inhibitors" Oral presentation at the 213th National ACS Meeting in San Francisco, CA, (Med Chem section), April 1997.

4. "Design and Synthesis of Novel Conformationally Restricted HIV Protease Inhibitors" Poster presentation at the 50th Gordon Conference on Medicinal Chemistry, Aug 1997.

5. "Structural Basis For Specificity of Pyrimidinylimidazole Inhibitors of p38 MAP Kinase" Oral presentation at the 4th SRI Anti-Inflammatory Drug Discovery Summit, Feb 1998

6. "Novel P38 MAP Kinase Inhibitors" Poster at the 51st Gordon Conference on Medicinal Chemistry, Aug 1998

7. "Design of p38 MAP Kinase Inhibitors for IL-1 and TNF Mediated Diseases" Invited speaker at the 2nd SRI TNF Conference Feb 1999

8. "Discovery of VX-745: A Novel p38 MAP Kinase Inhibitor. Invited presentation at the 52th Gordon Conference on Medicinal Chemistry, Aug 1999

9. Session Chair for the 4th International Symposium on Medicinal Chemistry of Neurodegenerative Diseases. Session Chair on Neuronal Apoptosis, Jan 2000

10. "Discovery of VX-745: A Novel p38 MAP Kinase Inhibitor. Invited presentation at the 27th National ACS Medicinal Chemistry Symposium, June 2000

11. "Discovery of VX-745: A Novel p38 MAP Kinase Inhibitor. Invited presentation at the 10th International IRA Meeting September 2000.

REFERENCES AVAILABLE UPON REQUEST

VPI/96-16 CIP 2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Deepak Rao
Group Art Unit	:	1624
Applicants	:	Guy W. Bemis et al.
Serial No.	:	09/336,266
Filed	:	June 18, 1999
For	:	INHIBITORS OF P38

New York, New York
March 8, 2001

Hon. Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF GUY W. BEMIS UNDER 37 C.F.R. § 1.132

I, GUY W. BEMIS, a citizen of the United States of America, residing at 256 Appleton Street, Arlington, MA (US), hereby declare that:

1. I am one of the named inventors of the above-identified patent application.

2. I received a B.S. in 1985 from the Pennsylvania State University, University Park, Pennsylvania in 1985. In 1991, I received a Ph.D. in Organic Chemistry from the University of Illinois at Champaign-Urbana,

Champaign, Illinois. After receiving my Ph.D, I did post-doctoral work concentrating on computational chemistry for 2 years at the University of California at San Francisco. A copy of my curriculum vitae is attached as **Exhibit 1**.

3. Since joining Vertex in 1993, my work has been devoted to computer-assisted molecular drug design. In particular, I have modeled a variety of pharmaceutical target molecules in three-dimensional space using computer molecular modeling programs, and have then studied spatial and energetic constraints between those targets and potential interacting molecules. Over the last 4 years, I have worked on using crystal structures of protein kinases to design and select compounds that can interact favorably with these protein kinases. In particular, I have worked on modeling complexes of p38 protein kinase and inhibitor compounds, based on their X-Ray crystal structures. I have co-authored 2 papers and have been a co-inventor on 3 published US patents related to modeling of these complexes.

4. I am familiar with the September 11, 2000 Office Action in the above-identified application. I understand that, in the Examiner's view, the specification does not allow one to extrapolate the activity of compounds embraced by the genus from the exemplified compounds. Specifically, the Examiner states that there is no reasonable

basis for assuming that the myriad of compounds embraced by the claims will all share the same physiological properties since they are so structurally dissimilar as to be chemically non-equivalent and there is no basis in the prior art (directed to p38 inhibitors) for assuming the same. See, the September 11, 2000 Office Action, page 4, ¶ 2.

I make this declaration to demonstrate that one of ordinary skill in the art would reasonably expect that the claimed compounds would have p38 inhibitory activity. I have based this conclusion upon molecular modeling data of the p38 kinase and certain representative model compounds of the claimed compounds.

5. Certain specific criteria determine an inhibitor's ability to bind to the active site of a target. For example, the internal strain energy for the inhibitor should be minimal when held in the binding conformation; there should not be any steric clashes between the atoms of the inhibitor and the amino acids at the enzyme active site; and there should be substantial attractive interactions between inhibitor atoms and enyzme atoms (e.g., hydrophobic interactions or hydrogen-bonds). Molecular modeling allows the evaluation of each of these criteria for a given inhibitor. Based on the molecular modeling results discussed below, one of ordinary skill in the art would reasonably

expect that the claimed compounds of the present application would inhibit p38.

6. I have attached hereto Exhibit B, Tables 1-4 showing representative compounds of formulae (Ie), (If), (Ig) and (Ih). I have analyzed these molecules using the QUANTA98 version 98.1111 and CHARMm version 25.2 revision 98.0731 (Molecular Simulations Inc.) and using proprietary X-Ray crystal structures of complexes of p38 and various inhibitors.

7. Table 1, Exhibit B shows eight representative compounds that fall within the genus of formulae (Ig) and (Ih). These eight compounds are characterized by a diverse group of Q_2 rings attached to the heterocyclic core ring system. The group of Q_2 rings ranges from indole (see, e.g., compounds 11 and 12), benzofuran (see, e.g., compound 13), benzothiophene (see, e.g., compound 14), pyrazole (see, e.g., compound 15), thiophene (see, e.g., compounds 16 and 17) and naphthalene (see, e.g., compound 18). Columns three and six in Table 1, Exhibit B show the corresponding kinase IC_{50} values, whereby the IC_{50} value gives the concentration of an inhibitor compound required to inhibit the p38 activity by 50%*. The eight compounds displayed in Table 1, Exhibit B, although

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* The p38 inhibition essay is described at page 95 of the specification as filed.

structurally dissimilar with respect to Q_2, are all inhibitors of p38. Therefore, the compounds provided in Table 1, Exhibit B demonstrate that compounds having a variety of ring systems for Q_2 have p38 inhibitory activity.

8. My colleagues at Vertex and I have obtained X-Ray crystal structure data of complexes of p38 and each of compound nos. 21 and 22. Compound no. 21 is representative of formula (Ig) and compound no. 22 is representative of formula (Ih). See, Table 2, Exhibit B. These X-Ray crystal structure data show that the Q_2 ring of each compound occupies the same pocket of p38, thus confirming that the Q_2 ring is structurally analogous for compounds of both formulae (Ig) and (Ih). Therefore, one having ordinary skill in the art would reasonably expect that the Q_2 ring of other compounds of formulae (Ig) and (Ih) would occupy the same pocket of p38 kinase. Further, my molecular modeling data of representative compounds of formulae (Ie) and (If) have shown that the Q_2 ring of these compounds also would bind to the same pocket of p38 as the Q_2 ring of compounds of formulae (Ig) and (Ih). See, compounds nos. 41-42 in Table 4, Exhibit B. X-Ray crystal structure data have shown that the Q_2 ring in formulae (Ig) and (Ih) occupies the pocket of the p38 kinase that includes amino acids Val-38, Leu-104, Leu-75, Leu-86, Thr-106, Ile-84, and Lys-53. My molecular modeling data show that the aromatic

ring system of Q_2 provides attractive interactions (which are mainly hydrophobic) with the above identified amino acids of the p38 kinase. These attractive interactions in general are a prerequisite for high p38 inhibitory activity. See, Table 1, Exhibit B. Therefore, given this data, one having ordinary skill in the art would reasonably expect that the claimed compounds of formulae (Ie), (If), (Ig) and (Ih) would have similar attractive interactions between their Q_2 rings and p38 kinase.

9. X-Ray crystal structure data of compound 22 shown in Table 2, Exhibit B also demonstrates that the Q_1 ring occupies a pocket of the p38 kinase that includes the amino acids Val-30, Tyr-35, Gly-110, Ala-111 and Asp-112. X-Ray crystal structure data of compound 21 (see, e.g., Table 2, Exhibit B) demonstrate that its Q_3 ring binds to the same pocket.

10. My colleagues at Vertex have synthesized and tested compounds of formula (Ih) wherein Q_1 is a substituted phenyl ring. See, e.g., compound nos. 401-410 and 412, in Table 5 of the specification as originally filed at pages 50-51. These compounds are p38 inhibitors with IC_{50} values ranging from 1.0 μM to <0.1 μM. Furthermore, we have synthesized and tested a compound of formula (Ih) wherein Q_1 is a benzo[1,3]dioxole ring. See, e.g., compound no. 31 in Table 3,

Exhibit B, which is also displayed as compound no. 411 in Table 5 of the specification as filed at page 51. This above compound exhibits an IC_{50} value of 0.02 μM.

In addition to the compounds shown in the instant application, we have modeled representative compounds that fall within the genus of formula (Ih), wherein Q_1 is a diverse range of aromatic carbocyclic or heterocyclic rings. These model compounds are displayed in Table 3, Exhibit B. See, e.g., compound nos. 31-36. The group of Q_1 rings ranges from chloro-benzo[1,3]-dioxole (see, e.g., compound no. 31), naphthalene (see, e.g., compound nos. 32 and 33), chloro-pyrrole (see, e.g., compound no. 34), chlorobenzofuran (see, e.g. compound no. 35) and methyl-indole (see, e.g. compound no. 36).

Computer modeling data obtained for compound nos. 31-36 show that the Q_1 rings of these model compounds have the following characteristics: a) little internal strain energy, b) no steric clashes with amino acids at the site in the p38 kinase where the Q_1 ring binds and c) substantial hydrophobic contact with the hydrophobic surfaces of the p38 kinase pocket, which include Tyr-35, Gly-110, Ala-111, Val-30 and Asp-112. These characteristics are typical for compounds with p38 inhibitory activity. Based on this computer modeling data, I believe that compound nos. 32-36 would bind to the p38

kinase and have inhibitory activity. See, paragraph 8, *supra*. Further, I reasonably believe that the Q_1 rings encompassed by the claimed compounds would interact in the same way with p38 kinase and would, therefore, have inhibitory activity. In addition, because ring Q_3 of compounds of formula (Ig) occupies the same pocket of p38 kinase as ring Q_1 of compounds of formula (Ih), and rings Q_1 and Q_3 are similar to one another, I reasonably expect that the claimed compounds of formula (Ig) are p38 inhibitors. See, paragraph 9, *supra*.

11. Modeling studies have also shown that ring Q_1 in compounds of formula (If) and ring Q_3 in compounds of formula (Ie) occupy the same pocket of the p38 kinase as ring Q_1 of compounds of formula (Ih). Table 4, Exhibit B shows two representative compounds that fall within the genus of formulae (If) (see, e.g., compound no. 41) and (Ie) (see, e.g., compound no. 42). Ring Q_1 and ring Q_3 of the above formulae interact with amino acids of the p38 kinase that include Val-30, Tyr-35, Gly-110, Ala-111 and Asp-112 as described in paragraph 8, *supra*. Given this data, one having ordinary skill in the art would reasonably expect that the claimed compounds of formulae (If) and (Ie) would have similar attractive interactions between their Q_1 and Q_3 rings and p38 kinase. Therefore, I reasonably believe that the Q_1 and Q_3

rings encompassed by the claimed compounds would interact in the same way with p38 kinase and would, therefore, have inhibitory activity.

12. Therefore, for the reasons presented above in paragraphs 7-11, I believe that one having ordinary skill in the art would reasonably expect that the claimed compounds of formulae (Ie), (Ig), (If) and (Ih) would have attractive interactions between their Q_1 or Q_3 rings and their Q_2 rings with the p38 kinase and thus, would have p38 inhibitory activity.

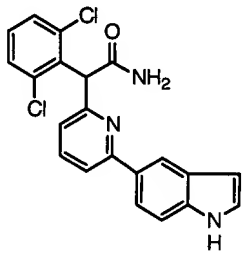
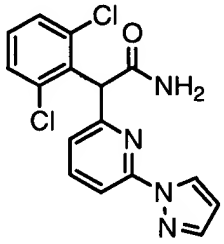
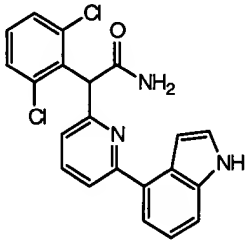
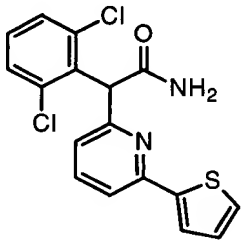
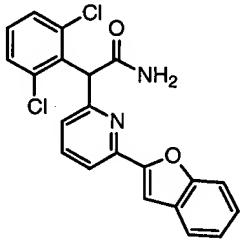
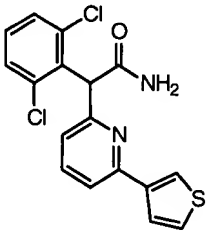
13. I declare further that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that such willful false statements may jeopardize the validity of this application and any patent issuing thereon.

Guy W. Bemis

Signed this 8th day of March, 2001
at Cambridge, MA, USA.

EXHIBIT B

Table 1: Compounds of formulae (Ig) and (Ih)

No.	Structure	IC_{50} (μM)	No.	Structure	IC_{50} (μM)
11 (Cpd. No. 372, Table 4 of spec.)		+	15		+
12		++	16 (Cpd. No. 317, Table 4 of spec.)		++
13 (Cpd. No. 355, Table 4 of spec.)		+	17 (Cpd. No. 318, Table 4 of spec.)		++

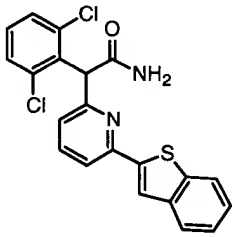
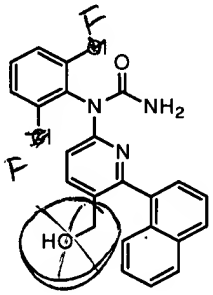
14 (Cpd. No. 356, Table 4 of spec.)		+	18 ~~NOT IN SCOPE~~		~~0.044~~ 0.024

Table 2: Compounds of formulae (Ig) and (Ih)

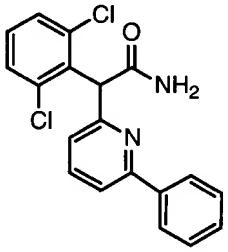
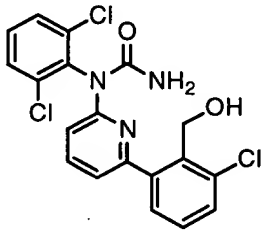
No.	Structure	No.	Structure
~~Modeled Compound~~ 21	 VRT-023109	~~Modeled Compound~~ 22	 VRT-033724
0.072			0.006

Table 3: Compounds of formula (Ih)

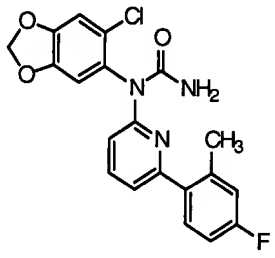
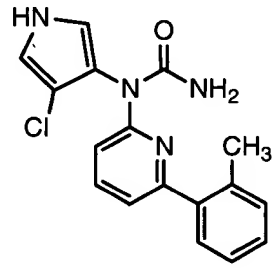
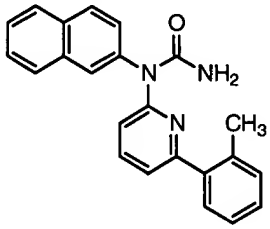
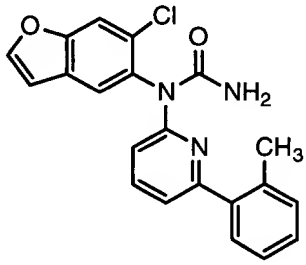
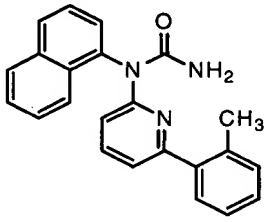
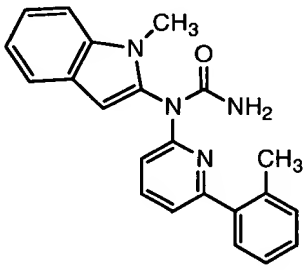
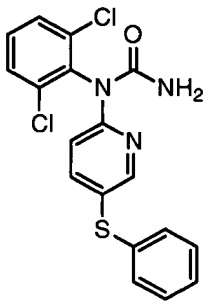
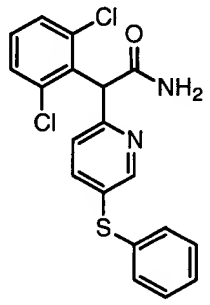
No.	Structure	IC_{50} (μM)	No.	Structure
31		+++	Modeled Compound 34	
Modeled Compound 32			Modeled Compound 35	
Modeled Compound 33			Modeled Compound 36	

Table 4: Compounds of formulae (If) and (Ie)

No.	Structure	IC_{50} (μM)	No.	Structure	IC_{50} (μM)
41		++	42		

Exhibit

analysis of databases and design of combinatorial chemistry lead discovery libraries. Algorithm development for analysis of the structures of drug molecules.

1991-1993
University of California at San Francisco
Postdoctoral researcher

Development of new molecular shape descriptor methods for lead discovery. Lead discovery for HIV protease enzyme.

1985-1991
University of Illinois, Urbana-Champaign, Illinois
Graduate Student/ Teaching Assistant

Computational design, organic synthesis, and kinetic evaluation of inhibitors for chymotrypsin-like serine proteases. Teaching assistant for Organic synthesis laboratory.

Summer 1985
Rohm & Haas, Spring House, Pennsylvania
Synthesis and polymerization of methacrylate-based monomers.

Published Papers:

Bemis GW, Murcko MA, *Properties of known drugs. 2. Side chains.*, J. Med. Chem., 42 (25): 5095-9 (1999)

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Education:

Post-Doctoral 1991-1993.
University of California, San Francisco
(with Professor Irwin D. Kuntz)

Ph.D. in Organic Chemistry 1991.
University of Illinois, Urbana-Champaign
(with Professor John A. Katzenellenbogen)

B.S. (with honors) 1985.
The Pennsylvania State University
(with Professor Roy A. Olofson)

Experience:

1993-present
Vertex Pharmaceuticals, Cambridge, Massachusetts
Intellectual Assets Head , Multi-Kinase Project 2000 - present
Senior Staff Investigator, Co-project head p38 research 1997-2000
Staff Scientist 1996-1997
Scientist 1993-1996

Head of "Intellectual Assets" team for Vertex multi-kinase project. This team will has created and implemented the IP strategies for kinase drug design.

Co-project head (and molecular modeler) for second generation p38 research program. Computational chemistry-based lead discovery and lead optimization for MAP kinases, ICE (and other caspases), IMPDH, and hemoglobin disorders. Molecular diversity-based

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Maryanoff BE, McComsey DF, Almond HR, Mutter MS, **Bemis GW**, Whittle RR, Olofson RA, *Dramatic Reversal of Diastereoselectivity in an N-Acyliminium Ion Cyclization Leading to Hexahydropyrrolo[2,1-a]isoquinolines.* A Case of Competing Steric Interactions, J Org Chem 51, 1341-1346 (1986)

Bemis GW, Whittle RR, Mayo SL, Olofson RA, *1,3,4,6-Tetramethyl-1,4-dihydro-1,2,4,5-tetrazine, C6H12N4*, Acta Cryst C40, 2076-2078 (1984)

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US6147080 Inhibitors of p38 , Vertex Pharmaceuticals Incorporated , Inventors: **Bemis, Guy W.** ;Salituro, Francesco Gerald ;Duffy, John Patrick ;Harrington, Edmund Martin, Serial No. 862925 , Filed 19970610 , Issued 20001114

US6103711 Inhibitors of interleukin-1.beta. converting enzyme,Vertex Pharmaceuticals, Incorporated , Inventors: **Bemis, Guy W.** ;Golec, Julian M. C. ;Lauffer, David J. ;Mullican, Michael D. ;Murcko, Mark A. ;Livingston, David J., Serial No. 465216 , Filed 19950605 , Issued 20000815

US6093742 Inhibitors of p38, Vertex Pharmaceuticals, Inc., Inventors: Salituro, Francesco Gerald ;**Bemis, Guy W.** ;Green, Jeremy ;Kofron, James L., Serial No. 884160 , Filed 19970627 , Issued 20000725

US5656627, Inhibitors of interleukin-1.beta. converting enzyme, Vertex Pharmaceuticals, Inc., Inventors: **Bemis, Guy W.** ;Golec, Julian M. C. ;Lauffer, David J. ;Mullican,

Michael D. ;Murcko, Mark A. ;Livingston, David J., Serial No. 405581 , Filed 19950317, Issued 19970812

US5716929, Inhibitors of interleukin-1.beta. converting enzyme, Vertex Pharmaceuticals, Inc. Inventors: **Bemis, Guy W.** ;Golec, Julian M. C. ;Lauffer, David J. ;Mullican, Michael D. ;Murcko, Mark A. ;Livingston, David J, Serial No. 464964 , Filed 19950605 , Issued 19980210

US5756466, Inhibitors of interleukin-1.beta. converting enzyme, Vertex Pharmaceuticals, Inc., Inventors: **Bemis, Guy W.** ;Golec, Julian M. C. ;Lauffer, David J. ;Mullican, Michael D. ;Murcko, Mark A., Serial No. 261452 , Filed 19940617 , Issued 19980526

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US5807876, Inhibitors of IMPDH enzyme, Vertex Pharmaceuticals Incorporated, Inventors: Armistead, David M. ;Badia, Michael C. ;**Bemis, Guy W.** ;Bethiel, Randy S. ;Frank, Catharine A. ;Novak, Perry M. ;Ronkin, Steven M. ;Saunders, Jeffrey O., Serial No. 636361 , Filed 19960423 , Issued 19980915

US5843904, Inhibitors of interleukin-1.beta.converting enzyme, Vertex Pharmaceuticals, Inc., Inventors: **Bemis, Guy W.** ;Duffy, John P. ;Fridman, Wolf Herman ;Golec, Julian M. C. ;Livingston, David J. ;Mullican, Michael D. ;Murcko, Mark A. ;Zelle, Robert E., Serial No. 575648 , Filed 19951220 , Issued 19981201

US5847135, Inhibitors of interleukin-1.beta. converting enzyme, Vertex Pharmaceuticals, Incorporated , Inventors: **Bemis, Guy W.** ;Golec, Julian M. C. ;Lauffer, David J. ;Mullican, Michael D. ;Murcko, Mark A. ;Livingston, David J., Serial No. 440898 , Filed 19950525 , Issued 19981208

US5874424, Inhibitors of interleukin-1.beta. converting enzyme, Vertex Pharmaceuticals Incorporated, Inventors: Batchelor, Mark James ;Bebbington, David ;**Bemis, Guy W.** ;Fridman, Wolf Herman ;Gillespie, Roger John ;Golec, Julian M. C. ;Lauffer, David J. ;Livingston, David J. ;Matharu, Saroop Singh ;Mullican, Michael D. ;Murcko, Mark A. ;Murdoch, Robert ;Zelle, Robert E, Serial No. 598332 , Filed 19960208 , Issued 19990223

US5945407, Methods and compositions using butyrate esters of threitol, Vertex Pharmaceuticals, Incorporated, Inventors: **Bemis, Guy W.** ;Chaturvedi, Pravin R., Serial No. 015811 , Filed 19980129 , Issued 19990831

US5945418, Inhibitors of p38, Vertex Pharmaceuticals Incorporated, Inventors: **Bemis, Guy W.** ;Salituro, Francesco Gerald ;Duffy, John Patrick, Serial No. 822373 , Filed 19970320 , Issued 19990831

US5973111, Inhibitors of interleukin-1.beta. converting enzyme, Vertex Pharmaceuticals, Inc., Inventors: **Bemis, Guy W.** ;Golec, Julian M. C. ;Lauffer, David J. ;Mullican, Michael D. ;Murcko, Mark A. ;Livingston, David J., Serial No. 828941 , Filed 19970328 ,Issued 19991026

US6008217, Inhibitors of interleukin-1.beta. converting enzyme, Vertex Pharmaceuticals Incorporated, Inventors: Batchelor, Mark James ;Bebbington, David ;**Bemis, Guy W.** ;Fridman, Wolf Herman ;Gillespie, Roger John ;Golec, Julian M. C. ;Lauffer, David J. ;Livingston, David J. ;Matharu, Saroop Singh ;Mullican, Michael D. ;Murcko, Mark A. ;Murdoch, Robert ;Zelle, Robert E., Serial No. 575641 , Filed 19951220 , Issued 19991228

US6025147, Inhibitors of interleukin-1 .beta. converting enzyme, Vertex Pharmaceuticals, Inc., Inventors: **Bemis, Guy W.** ;Golec, Julian M. C. ;Lauffer, David J. ;Mullican, Michael D. ;Murcko, Mark A., Serial No. 460973 , Filed 19950605 , Issued 20000215

US6054472, Inhibitors of IMPDH enzyme, Vertex Pharmaceuticals, Incorporated Inventors: Armistead, David M. ;Badia, Michael C. ;**Bemis, Guy W.** ;Bethiel, Randy S. ;Frank, Catharine A. ;Novak, Perry M. ;Ronkin, Steven M. ;Saunders, Jeffrey O. Serial No. 832165 , Filed 19970402 , Issued 20000425

WO0075118 Inhibitors Of C-Jun N-Terminal Kinases (Jnk) Vertex Pharmaceuticals Incorporated, Inventors: ;Salituro, Francesco ;**Bemis, Guy** ;Green, Jeremy ;Fejzo, Jasna ;Xie, Xiaoling , Application No. US0015248 , Filed 20000602 , A1 Published 20001214 ,

WO0064872, Inhibitors Of C-Jun N-Terminal Kinases (Jnk), Vertex Pharmaceuticals Incorporated, Inventors: ;Salituro, Francesco, Gerald ;**Bemis, Guy, W.** ;Wilke, Susanne ;Green, Jeremy ;Cao, Jingrong ;Gao, Huai ;Harrington, Edmund, Martin, Application No. US0010866 , Filed 20000421 , A1 Published 20001102 ,

WO0036096, Crystallized P38 Complexes, Vertex Pharmaceuticals Incorporated, Inventors: ;Bellon, Steven ;**Bemis, Guy** ;Wilson, Keith ;Fitzgibbon, Matthew , Application No. US9929096 , Filed 19991208 , A1 Published 20000622

WO0017204 , Inhibitors Of P38,Vertex Pharmaceuticals Incorporated, Inventors: Salituro, Francesco ;**Bemis, Guy** ;Gao, Huai, Application No. US9921567 , Filed 19990916 , A1 Published 20000330

WO0017175, Inhibitors Of P38, Vertex Pharmaceuticals Incorporated, Inventors: Salituro, Francesco ;**Bemis, Guy** ;Cochran, John, Application No. US9921337 , Filed 19990916 , A1 Published 20000330

WO9535308, Inhibitors Of Interleukin-1'beta' Converting Enzyme, Vertex Pharmaceuticals Incorporated, Inventors: **Bemis, Guy, W.** ;Golec, Julian, M., C. ;Lauffer, David, J. ;Mullican, Michael, D. ;Murcko, Mark, A. ;Livingston, David, J., Application No. US9507617 , Filed 19950616 , A1 Published 19951228

WO9716180, Methods And Compositions Using Butyrate Esters Of Threitol, Vertex Pharmaceuticals Incorporated, Inventors: **Bemis, Guy, W.** ;Chaturvedi, Pravin, R., Application No. US9617244 , Filed 19961030 , A1 Published 19970509

WO9722618, Inhibitors Of Interleukin-1'beta' Converting Enzyme, Vertex Pharmaceuticals Incorporated, Inventors: **Bemis, Guy, W.** ;Duffy, John, P. ;Fridman, Wolf, Herman ;Golec, Julian, M., C. ;Livingston, David, J. ;Mullican, Michael, D. ;Murcko, Mark, A. ;Zelle, Robert, E., Application No. US9620370 , Filed 19961220 , A1 Published 19970626

WO9722619, Inhibitors Of Interleukin-1'beta' Converting Enzyme, Vertex Pharmaceuticals Incorporated, Inventors: Batchelor, Mark, J. ;Bebbington, David ;**Bemis, Guy, W.** ;Fridman, Wolf, Herman ;Gillespie, Roger, J. ;Golec, Julian, M., C. ;Gu, Yong ;Lauffer, David, J. ;Livingston, David, J. ;Matharu, Saroop, S. ;Mullican, Michael, D. ;Murcko, Mark, A. ;Murdoch, Robert ;Nyce, Philip, L. ;Robidoux, Andrea, L., C. ;Su, Michael ;Wannamaker, M., Woods ;Wilson, Keith, P. ;Zelle, Robert, E., Application No. US9620843 , Filed 19961220 , A2 Published 19970626

WO9740028, Urea Derivatives As Inhibitors Of IMPDH Enzyme, Vertex Pharmaceuticals Incorporated, Inventors: Armistead, David, M. ;Badia, Michael, C. ;**Bemis, Guy, W.** ;Bethiel, Randy, S. ;Frank, Catharine, A. ;Novak, Perry, M. ;Ronkin, Steven, M. ;Saunders, Jeffrey, O., Application No. US9706623 , Filed 19970421 , A1 Published 19971030

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WO9857155, Methods for Identifying Drug Cores, Vertex Pharmaceuticals Incorporated, Inventors: Moore, Jonathan ;**Bemis, Guy, William** ;Lepre, Christopher, A. ;Fejzo, Jasna ;Peng, Jeffrey, Weilee ;Wilson, Keith, Phillip; Murcko, Mark, Andrew, Application No. US9812393 , Filed 19980615 , A1 Published 19981217

WO9900357, Inhibitors of p38, Vertex Pharmaceuticals Incorporated, Inventors: Salituro, Francesco, Gerald ;**Bemis, Guy, W.** ;Green, Jeremy ;Kofron, James, L., Application No. US9813496 , Filed 19980629 , A1 Published 19990107

WO9947545, Inhibitors of Caspases, Vertex Pharmaceuticals Incorporated, Inventors: Wannamaker, Marion, W. ;**Bemis, Guy, W.** ;Charifson, Paul, S. ;Lauffer, David, J. ;Mullican, Michael, D. ;Murcko, Mark, A. ;Wilson, Keith, P. ;Janetka, James, W. ;Davies, Robert, J. ;Grillot, Anne-Laure ;Shi, Zhan ;Forster, Cornelia, J. Application No. US9905919 , Filed 19990319 , A2 Published 19990923

WO9958502, Heterocyclic Inhibitors of p38, Vertex Pharmaceuticals Incorporated, Inventors: Salituro, Francesco ;Galullo, Vincent ;Bellon, Steven ;**Bemis, Guy** ;Cochran, John, Application No. US9910291 , Filed 19990511 , A1 Published 19991118

WO9964400, Inhibitors Of p38, Vertex Pharmaceuticals Incorporated , Inventors: Salituro, Francesco ;**Bemis, Guy** ;Cochran, John, Application No. US9912951 , Filed 19990611 , A1 Published 19991216

EP0784628, Inhibitors Of Interleukin-1-g(b) Converting Enzyme, Vertex Pharmaceuticals Incorporated, Inventors: **Bemis, Guy W.** ;Golec, Julian M.C. ;Lauffer, David J. ;Mullican, Michael D. ;Murcko, Mark A. ;Livingston, David J., Application No. EP95925257 , Filed 19950616 , A1 Published 19970723

EP0862426, Methods And Compositions Using Butyrate Esters Of Threitol, Vertex Pharmaceuticals Incorporated, Inventors: **Bemis, Guy, W.** ;Chaturvedi, Pravin, R., Application No. EP96937773 , Filed 19961030 , A1 Published 19980909

EP0869967, Inhibitors Of Interleukin-1$g(b) Converting Enzyme, Vertex Pharmaceuticals Incorporated, Inventor(s): ;Batchelor, Mark, J. ;Bebbington, David ;**Bemis, Guy, W.** ;Fridman, Wolf, Herman ;Gillespie, Roger, J. ;Golec, Julian, M., C. ;Gu, Yong ;Lauffer, David, J. ;Livingston, David, J. ;Matharu, Saroop, S. ;Mullican, Michael, D. ;Murcko, Mark, A. ;Murdoch, Robert ;Nyce, Philip, L. ;Robidoux, Andrea, L., C. ;Su, Michael ;Wannamaker, M., Woods ;Wilson, Keith, P. ;Zelle, Robert, E., Application No. EP96945318 , Filed 19961220 , A2 Published 19981014

EP0876395, Inhibitors Of Interleukin-1$g(b) Converting Enzyme, Vertex Pharmaceuticals Incorporated, Inventors: **Bemis, Guy, W.** ;Duffy, John, P. ;Fridman, Wolf, Herman ;Golec, Julian, M., C. ;Livingston, David, J. ;Mullican, Michael, D., ;Murcko, Mark, A. ;Zelle, Robert, E., Application No. EP96945237 , Filed 19961220 , A1 Published 19981111

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Presentations:

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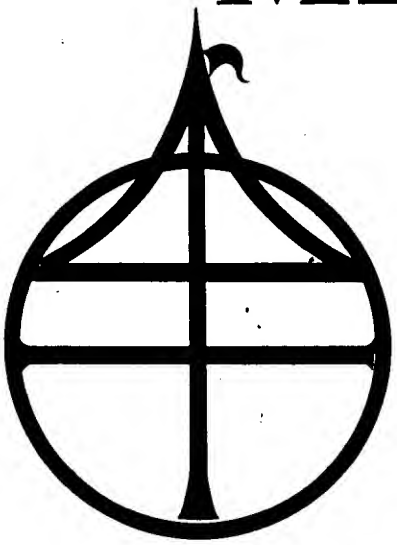

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PART XIV

ONCOLOGY

154 INTRODUCTION

Joseph V. Simone

BACKGROUND

DEFINITIONS, INCIDENCE, AND MORTALITY. Cancer describes a class of diseases characterized by the uncontrolled growth of aberrant cells. Cancers kill by the destructive invasion of normal organs through direct extension and spread to distant sites via the blood, lymph, or serosal surfaces. The abnormal clinical behavior of cancer cells is often mirrored by biologic aberrations such as genetic mutations, chromosomal translocations, expression of fetal or other discordant ontologic characteristics, and the inappropriate secretion of hormones or enzymes. All cancers invade or metastasize, but each specific type has unique biologic and clinical features that must be appreciated for proper diagnosis, treatment, and study. About 1.2 million new cases of invasive cancer are diagnosed each year in the United States, and about 500,000 people die annually of the disease. Cancer is the second most deadly disease and is expected to surpass heart disease early in the twenty-first century to top that nefarious list. Over the past half century, the frequency of most cancers has been stable, but some dramatic changes have taken place (Fig. 154–1). Steady declines in stomach and uterine cancer have occurred, the latter undoubtedly due to routine cytologic screening for cervical cancer. The cause of the decline in stomach cancer is unknown. The most striking change has been the increases in lung cancer in both men and women, undoubtedly related to smoking. Other cancers with increasing mortality, particularly in the elderly, include melanoma, non-Hodgkin's lymphoma, and brain tumors. There have been speculations but little firm evidence to explain these changes. The overall mortality from cancer, particularly for those under age 65, has declined, primarily due to more effective therapy for cancers of fetal and hematopoietic origin that occur in the younger population. See Ch. 157 for more detailed treatment of cancer epidemiology.

ETIOLOGY AND PREVENTION. A broad array of agents can cause or directly contribute to a sequence of events or sensitize cells in such a way that cancer develops. The final common pathway in virtually every instance is a cellular genetic mutation that converts a well-behaved cellular citizen of the body into a destructive renegade that is unresponsive to the ordinary checks and balances of a normal community of cells. Promoters (oncogenes) and suppressors (like the retinoblastoma or *p53* gene) play a central role in many cases (see Ch. 156). Chemicals such as benzene and nitrosamines, physical agents such as gamma and ultraviolet radiation, and biologic agents such as the Epstein-Barr and hepatitis viruses contribute to carcinogenesis under certain circumstances. Evidence exists to link dietary factors to carcinogenesis; although not as clear as one would like, the evidence is strong enough to recommend diets low in fat and high in fiber. A sensible diet is based on grains, vegetables, and fruits, with smaller than the current average proportions of fat. Inherited susceptibilities are becoming more evident and probably play a key role in a significant number of cancers of the breast and colon. Down syndrome and the Li-Fraumeni syndrome are well-known harbingers of a substantial risk for developing cancer.

The single most important carcinogen in the United States and Europe is tobacco, because it causes or contributes to the development of about one-third of all cancers—primarily lung, esophageal, head and neck, and bladder. Less well appreciated is the contribution tobacco may make to causing breast, colon, and gastric cancer. Tobacco-related cancer is also important because it is preventable by the obvious, inexpensive, and 100% effective means of abstention. Although the total number of smokers in the United States has declined, through the skillful and irresponsible efforts of tobacco companies women smoke more than ever, adolescents continue to view smoking as socially chic, and the number of smokers in Asia and the third world countries is growing at an alarming rate. Cancer etiology and prevention are treated in more detail in Ch. 155.

EARLY DETECTION OF CANCER. When prevention of cancer is not possible because effective means are lacking, early detection is the next best strategy to reduce cancer mortality. The American Cancer Society (ACS) has recommended a series of cancer screening procedures for asymptomatic individuals (Table 154–1). Not all experts agree on the frequency or age ranges for employing such procedures, but the ACS recommendations are a well-considered and useful guide that, at the very least, indicates the cancers most amenable to clinically useful early detection by conventional techniques. An even more exciting development in this effort has been the emergence of genetic screening and counseling of families at high risk for developing cancer. Individuals at risk are identified largely by analysis of family pedigrees, and the increasing availability of the revolutionary tools of molecular biology can identify specific genetic mutations (see Ch. 156). As this is being written, the cloning of a mutated gene associated with a large minority of breast cancers appears imminent. It is certain that many such genes will be identified, focusing the cancer screening and early detection efforts more efficiently and productively on high-risk populations (see Ch. 155).

CANCER TUMOR GROWTH. While it is impossible to know the specific details of early *in vivo* tumor growth and the efficiency of tumor cell renewal of human cancer, clinical and laboratory observations have provided a reasonable conceptual framework. This framework should be used with caution, however, because it is certain that the intrinsic factors that control tumor growth and propagation are far more complex, episodic, and heterogeneous than we know, even within a single tumor mass. Furthermore, the stromal environment and neovascularization of tumors have become more central to our understanding of this process than heretofore. Nonetheless, the following description can be a useful reference point.

A tumor has reached the size of clinical detectability when it contains about 10^9 cells, weighing about 1 gram and occupying a volume of about 1 cc. A three-log increase to 10^{12} cells, 1 kg, and 1000 cc is often lethal. Below 10^9 cells, the tumor is usually undetectable, but it has already undergone at least 30 doublings, and only 10 further doublings will produce the 1 kg of tumor. This exercise illustrates how much has already occurred, with all the opportunities for the cancer to undergo advantageous mutation and metastasis, before clinical detection. Once the tumor has grown into the clinically evident range, it tends to grow progressively slower with increasing size. This deceleration of growth probably occurs because the tumor outgrows its blood supply, reaches anatomic boundaries, and responds to yet undiscovered feedback regulation from other members of the now larger and more heterogeneous mass of tumor cells. Thus cancers probably grow much like bacteria after inoculation into a favorable medium. The phases of bacterial growth describe a sigmoid curve (Fig. 154–2): an early lag phase of inapparent or slow growth followed by exponential growth. Growth then slows when new cell production and cell

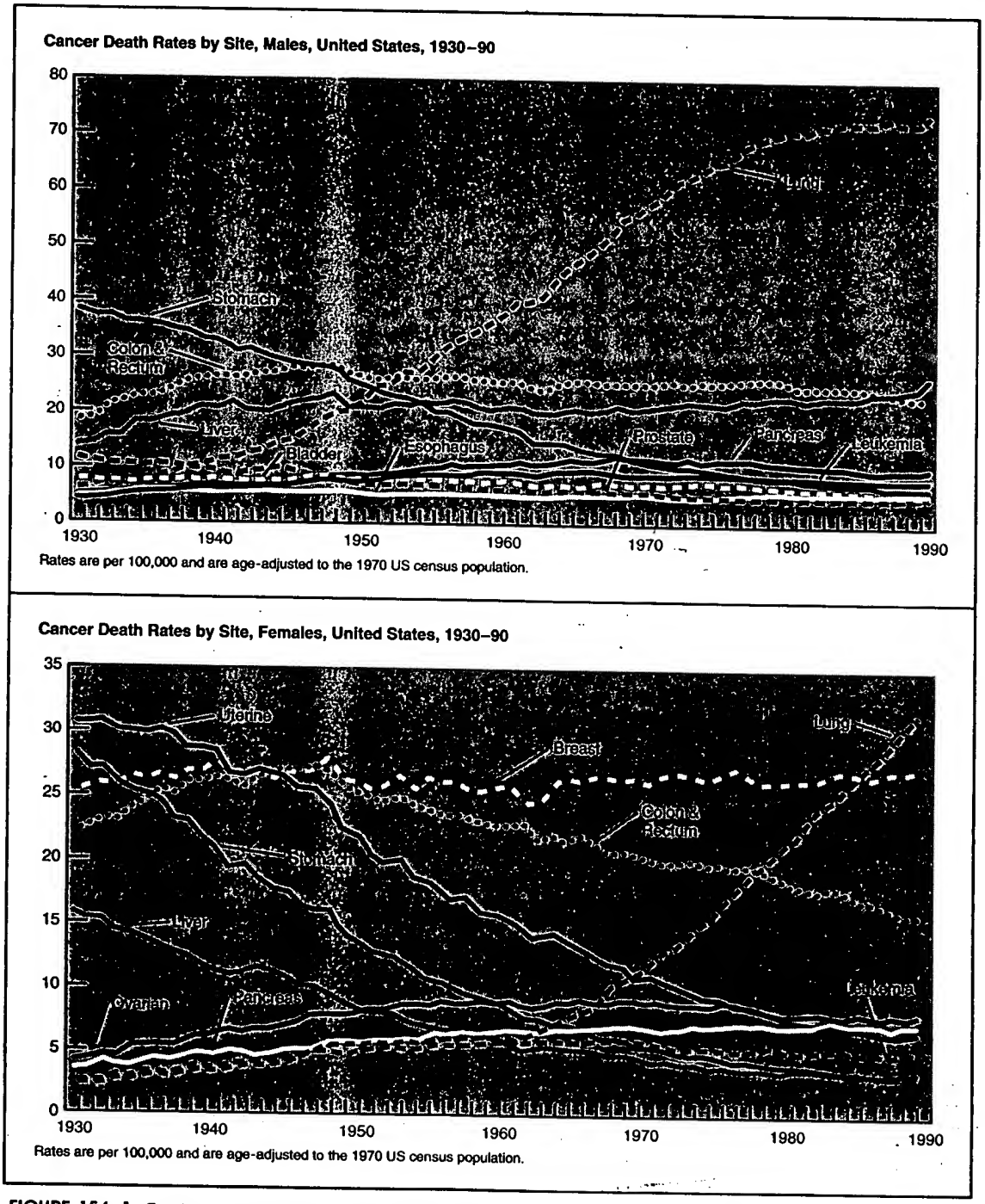

FIGURE 154–1. Cancer death rates in the United States. (From Cancer Facts and Figures—1994. Atlanta, American Cancer Society, 1994.)

death are nearly equal, with the latter phase in culture due to crowding and inadequate nutrients. Of course, in bacteria as well as cancers, the specific growth characteristics differ among types as well as within types that have developed subpopulations of mutant clones.

Most chemotherapy acts by damaging DNA, so it tends to be most effective in rapidly growing tumors such as acute leukemia, lymphomas, and testicular cancers. Also, after gross surgical removal, residual cancer cells may grow more rapidly and be more sensitive to subsequent ("adjuvant") chemotherapy. The sensitivity or resistance to chemotherapy or radiation, however, probably has as much or more to do with the specific biochemical and metabolic features of the cancer cell as with its growth characteristics (see Ch. 165).

MANAGEMENT OF THE PATIENT WITH CANCER

Oncology has been transformed over the past 40 years. From a diverse set of orphan diseases usually managed by surgeons alone and viewed with despair by most physicians, it has become a complex and exciting discipline that draws its strength from the essential partnership of specialists in medicine, surgery, pediatrics, pathology, radiation oncology, diagnostic imaging, psychiatry, and others. This remarkable evolution can be credited to therapeutic successes and biologic advances that could not be imagined in the early 1950's. At its best, oncology has pointed the way to an understanding of the biologic variability of cancer and the success that is possible with a coordinated multimodal approach to therapy.

GOALS. The oncologist—that is, anyone who seriously and expertly assumes responsibility for the management of patients with cancer—should have three sets of goals: therapeutic, human, and scientific. The initial therapeutic goal is to cure patients and return them to a normal place in society. This should be attempted in virtually all cancers, even when the likelihood of cure is small. It requires an attitude of reasonable hope and determination as well as a willingness to attempt difficult, dangerous, and sometimes daring approaches to fundamentally resistant diseases. If after a reasonable

TABLE 154–1. SUMMARY OF AMERICAN CANCER SOCIETY RECOMMENDATIONS FOR THE EARLY DETECTION OF CANCER IN ASYMPTOMATIC PEOPLE

Test or Procedure	Population		
	Sex	*Age*	*Frequency*
Sigmoidoscopy, preferably flexible	M & F	50 and over	Every 3–5 years
Fecal occult blood test	M & F	50 and over	Every year
Digital rectal examination	M & F	40 and over	Every year
Prostate examination*	M	50 and over	Every year
Papanicolaou test	F	All women who are or who have been sexually active, or have reached age 18, should have an annual Papanicolaou test and pelvic examination. After a woman has had three or more consecutive satisfactory normal annual examinations, the Papanicolaou test may be performed less frequently at the discretion of her physician.	
Pelvic examination	F	18–40	Every 1–3 years with Papanicolaou test
		Over 40	Every year
Endometrial tissue sample	F	At menopause, if at high risk†	At menopause and thereafter at the discretion of the physician
Breast self-examination	F	20 and over	Every month
Breast clinical examination	F	20–40	Every 3 years
		Over 40	Every year
Mammography‡	F	40–49	Every 1–2 years
		50 and over	Every year
Health counseling and cancer checkup§	M & F	Over 20	Every 3 years
	M & F	Over 40	Every year

From Cancer Facts and Figures—1994. Atlanta, American Cancer Society, 1994.

* Annual digital rectal examination and prostate-specific antigen should be performed on men 50 years and older. If either is abnormal, further evaluation should be considered.

† History of infertility, obesity, failure to ovulate, abnormal uterine bleeding, or unopposed estrogen or tamoxifen therapy.

‡ Screening mammography should begin by age 40.

§ To include examination for cancers of the thyroid, testicles, prostate, ovaries, lymph nodes, oral region, and skin.

attempt permanent cure is not possible, the physician must not abandon the patient but should aim for a secondary goal, a long, qualitatively satisfactory remission. If this is no longer possible, the tertiary level of therapeutic intent is to obtain a remission of any kind and duration; however, at this stage and later, one is less willing to expose the patient to the possibility of serious side effects or long hospitalization. When the possibility of remission of any type becomes remote, the goal at the fourth level is to control the disease and symptoms by the judicious use of palliative therapeutic measures.

The objective in the final stage is terminal care, which is always difficult because it requires the admission that specific therapy is no longer of any value. The only goal now is to provide comfort. Instead of blood transfusions, antibiotics, or chemotherapeutic agents, the physician must use pain medications, sedation, psychosocial support, and other comfort measures with the thought of returning the patient to the home or other appropriate setting and to the support of family.

The human goals in oncology are inextricably linked with the therapeutic and scientific goals. Physicians, nurses, and other health care providers wish to cure patients or improve their conditions so that they may fulfill their human destiny as well as possible. This requires sensitivity to the particular needs of the patient and family and an understanding of the social environment from which they came and to which they must return. The physician must help them maintain their dignity, understand their weaknesses, and refuse to allow any frustration, animosity, or excessive friendship to develop and threaten good judgment and the best interests of the patient.

The use of scientific methods in oncology is only in its adolescence, and definitive treatment has been established for only a small proportion of the circumstances and types of cancers that can arise. Systematic protocol studies yield useful information about a new drug, a novel regimen, or a biologic feature. Presentation and criticism of one another's efforts in a collegial and scientific manner are essential to advancing the knowledge about a particular treatment. Physicians who manage a small number of patients per year cannot possibly have the background and support necessary to treat these complex diseases adequately. This task is best left to specialists who participate in active scientific programs and have the resources to deliver optimal clinical care. It is also important to understand the limitations of science and that at times no treatment is the best option.

DIAGNOSTIC PRINCIPLES. The first diagnostic principle is that adequate tissue must be obtained from the tumor to establish the specific diagnosis and subtype of cancer. The rare exceptions are instances in which a biopsy might be life-threatening and the anatomic location is virtually pathognomonic of a specific histology. Some brain tumors and anterior mediastinal tumors that compress the trachea and blood vessels are two notable examples. In the latter situation, often due to a lymphoma, steroids may reduce the tumor size and relieve symptoms before a biopsy is attempted. More often, an adequate sample must be obtained before therapy is started unless complete surgical excision is definitively diagnostic and therapeutic. Because management of each type and subtype of cancer is often distinctive, every effort must be made to obtain appropriate samples, even if therapy is delayed for a short time. A specific diagnosis is seldom a problem in the leukemias because bone marrow aspiration usually affords a ready answer; the solid tumors present the greater difficulty.

Cancer diagnosis may be challenging and urgent; an understanding of some of its unusual manifestations can be very helpful. Elsewhere in this text, sound guidance is provided on paraneoplastic syndromes (see Ch. 158), endocrine manifestations of cancer (see Ch. 159), cutaneous manifestations of cancer (see Ch. 161), and oncologic emergencies (see Ch. 163).

A second diagnostic principle is to establish the extent of the disease. In the leukemias, this can be readily accomplished by physical examination, routine laboratory tests, chest roentgenography, and examination of cerebrospinal fluid. With solid tumors, determining the extent of the disease, that is, the *stage* of the tumor, often involves major surgery and an extensive examination that uses diagnostic imaging techniques. A coordinated approach involving the surgeon and pathologist is crucial to determine the extent of tumor invasion; without this approach, one may lack essential information for planning treatment and for judging its success. Failure to detect

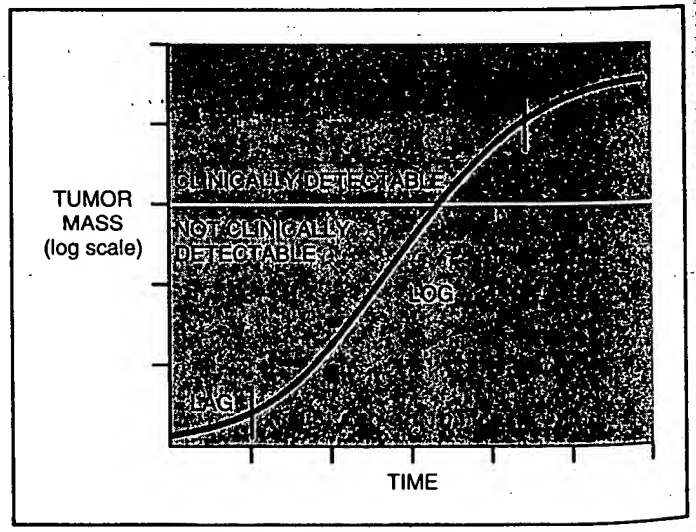

FIGURE 154–2. A schematic representation of the phases of growth of a cancer. After a period of inapparency (lag phase), growth tends to be logarithmic, followed by deceleration due to inadequate nutrients, competitive inhibition among cells, or a lack of neovascularization. (This resembles the growth of bacteria inoculated into a favorable medium.) The tumor has gone through many doublings before it becomes clinically apparent.

a tumor that has extended to regional lymph nodes can lead to undertreatment and a false impression that the local treatment, whether surgery or radiation therapy, was adequate. A simplified generic staging system is shown in Table 154–2. More detailed and specific staging systems have been developed for most cancers that take into account peculiar pathogenetic features, modes of spread, and potential curability. In addition, modern oncology demands an extensive biologic classification of leukemias and solid tumors, often requiring sophisticated scientific approaches not available a few years ago. This includes the use of monoclonal antibodies to determine the phenotype of lymphomas and leukemias, light and electron microscopy with special stains to determine the presence of glycogen, enzymes, or other substances that help to classify solid tumors, chromosomal analysis and modern molecular probes that identify unique characteristics of a disease, and responsible oncogenes, suppressor genes, and familial genes (see Ch. 156 and 158).

THERAPEUTIC PRINCIPLES. The first step in treatment is to know the patient. All pertinent information—medical, developmental, and social—must be sought before treatment is planned. The second step is to know the tumor: its usual behavior, usual rate of growth, mode of spread, whether it is local or systemic, and any features that may provide prognostic or therapeutic leads. Third, one must know the available therapies: not only the therapeutic modalities such as chemotherapy, radiation therapy, and surgery but also the skills and limitations of colleagues. Finally, one must know oneself: one's skills, experience, objectivity, and limitations. All these factors shape decisions concerning the patient. Treating patients with cancer is not easy; one must be prepared for losses as well as gains while keeping overall progress and success in mind.

As indicated above, clarity of intent—whether curative, palliative, or supportive—will avoid the confusion of approach and method. Treatment protocols—either research or "standard of care" regimens—are important tools in this regard because they allow strategies to be planned should any momentary decisions be necessary. Protocols are also more likely to provide useful conclusions from a study or experience, because a scientific question or a uniform approach has been formulated and data have been collected in a systematic manner. A protocol is, however, only a road map. The planned therapy may require adjustment if complications develop after treatment has begun. Although many of these adjustments can be anticipated and specified in the protocol, not every circumstance can be foreseen. A protocol is also intended to provide practical information that will lead to improved treatment of subsequent patients.

THERAPEUTIC MODALITIES. There are four principal therapeutic modalities for cancer. *Surgery* is the oldest and most definitive when the tumor is localized under the most favorable anatomic circumstances. For example, for a small tumor localized in the breast, the interior of one kidney, or the peripheral edge of the liver, surgery is usually definitive, curative, and leaves no undue side effects. For many solid tumors, however, surgery alone is inadequate because of local or distant spread. Surgery is also crucial in establishing the extent of a tumor. Considerable surgical skill and experience are required to approach a tumor that may or may not be resectable, achieve tumor-free margins, and obtain the necessary tissue without causing further dissemination.

Radiotherapy is most useful for localized tumors that cannot be resected at all or without serious morbidity and for tumors, such as Hodgkin's disease, that tend to spread to predictable contiguous sites. Therefore, a port of radiation can be enlarged beyond the known extent of the tumor and be quite effective. Unfortunately, radiotherapy can have serious side effects, especially in children who are growing and developing. Nonetheless, the skilled use of radiotherapy is an essential part of oncology; as with all modalities, its role changes depending on new knowledge about a particular tumor. The dosage of radiotherapy is based on an estimate of the dose absorbed by tumor, measured in equivalent units called "centigrays" (cGy) or "rads."

Chemotherapy was the first systemic treatment for any cancer. It most often consists of a combination of drugs, which is almost always more effective than the sequential use of single agents. Since tumors develop subpopulations of cells that differ in their sensitivity to antineoplastic drugs, combinations of agents destroy more cells more rapidly, thereby reducing the frequency of emergence of resistant clones. The mechanisms of action of common chemotherapeutic agents differ widely, although DNA damage is the common final pathway. Toxicity also differs among agents; myelosuppression and gastrointestinal disorders are the most common disturbances. Although toxicity is a concern, for many cancers the best therapeutic results depend on the intensity of the dosage; that is, effective agents given at higher doses over a shorter period are more efficacious than less intensive regimens. One must straddle the fine line between too much and too little.

Chemotherapy is used (1) as a definitive treatment, as in leukemia and some lymphomas; (2) as a principal form of treatment, as in testicular cancer and Ewing's sarcoma; or (3) as an adjuvant to another modality, such as amputation for osteosarcoma or surgical resection for breast or bowel cancer.

Biologic therapy for cancer includes, in addition to bone marrow transplantation, the newer uses of biologic response modifiers such as lymphokines or monoclonal antibodies and agents such as retinoic acid that may cause tumor cells to undergo differentiation and become harmless. These approaches, although still under development, show promise for the future.

The success of cancer therapy often depends on the skillful combination of two or more treatment modalities necessitating close cooperation of medical specialists. Failure to coordinate the effort may lead to the use of modalities in a useless or harmful sequence with an ineffective result.

Supportive care encompasses skilled general medical care. It includes management of infectious, metabolic, and cardiopulmonary disorders that frequently occur in patients undergoing aggressive treatment or surgical procedures. The judicious use of blood products is an essential part of supportive care, and infectious complications in the immunosuppressed patient must be anticipated. Because infections account for a large proportion of hospitalizations and deaths in patients with cancer, one cannot provide modern therapy without appropriate support from specialists in infectious diseases.

MEASURES OF SUCCESS. The measures of success in the treatment of patients with cancer are relatively simple, although not always precise. The first is survival without recurrence of tumor. Unfortunately, some malignancies recur many years after apparently successful control. An operative definition of cure, therefore, differs for each cancer. A patient who remains tumor-free for 2 years after completing therapy is probably cured if the tumor was neuroblastoma, lung cancer, acute myeloid leukemia, or lymphoblastic lymphoma. A much longer period would be needed to conclude a cure for breast cancer, Ewing's sarcoma, Hodgkin's disease, or acute lymphoblastic leukemia of childhood.

The second measure of success is resumption of a normal life pattern without sequelae from the disease or its treatment. The Karnofsky scale (Table 154–3) is a useful guide to measure "performance status." Unfortunately, late side effects, such as second malignancies, may occur 10 to 15 years after treatment is completed. A good estimate of success and failure is usually apparent in a few years, but long-term follow-up of patients is essential for definitive answers.

TABLE 154–2. SIMPLIFIED GENERIC CANCER STAGING SYSTEM

Stage 1	Localized. Usually confined to the organ of origin. Usually curable with locally effective measures such as surgery or irradiation.
Stage 2	Regional. Extends beyond organ of origin but remains nearby, in lymph nodes, for example. Often curable by local measures alone or in combination (surgery ± irradiation) or by a local modality with chemotherapy.
Stage 3	Extensive. Has extended beyond regional site of origin, crossing several tissue planes or extending more distantly via lymphatics or blood. Also may be confined to an organ or region, but be unresectable because of anatomic extent or location. This stage is used rather than stage 2 or stage 4 depending upon the usefulness of local and systemic treatment modalities and the likelihood of cure for that specific cancer.
Stage 4	Widely disseminated. Often involves the bone marrow or multiple distant organs. Rarely curable with current armamentarium.

TABLE 154–3. PERFORMANCE STATUS (KARNOFSKY SCALE)

	Criteria of Performance Status (PS)	
Able to carry on normal activity; no special care is needed	100	Normal; no complaints; no evidence of disease
	90	Able to carry on normal activity; minor signs or symptoms of disease
	80	Normal activity with effort; some signs or symptoms of disease
Unable to work; able to live at home and care for most personal needs; a varying amount of assistance is needed	70	Cares for self; unable to carry on normal activity or to do active work
	60	Requires occasional assistance but is able to care for most needs
	50	Requires considerable assistance and frequent medical care
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly	40	Disabled; requires special care and assistance
	30	Severely disabled; hospitalization is indicated although death not imminent
	20	Very sick; hospitalization necessary; active supportive treatment is necessary
	10	Moribund, fatal processes progressing rapidly
	0	Dead

PATIENT-FAMILY-PHYSICIAN RELATIONSHIP. Patients with cancer and their families face an extremely difficult time. They need a physician who is hopeful, truthful, compassionate, understanding, accessible, informative, and knowledgeable. Although cancer patients understand that several physicians and other professionals will be involved in their care, they prefer and need one physician who can assume ultimate responsibility for their myriad needs.

Patients should be told of plans and procedures in language that is understandable and appropriate. Some idea of the nature of cancer can be provided by analogy. For example, one may compare leukemia to the overgrowth of a farmer's field (bone marrow) by weeds (leukemia cells) that prevent the growth and export of crops (normal blood cells). Because the weeds cannot be removed manually from the marrow, chemicals are used to destroy the weeds and allow the crops to grow.

Physicians and family often mistakenly believe that the patient is only concerned with the possibility of death. In fact, patients are often equally or more concerned with the immediate implications of disease, for example, separation from family, pain, disfigurement, lengthy hospitalization, financial ruin, or missed time at work or school. Sensitive caregivers will understand and try to address these issues. Some patients and families become very knowledgeable about the disease and in fact may know as much as or more than physicians about certain details; this should be viewed as an asset that can aid the physician in management. Physicians, nurses, and other caregivers may become emotionally attached to a patient or the family. This need not be avoided as long as the necessary professional relationship and sound medical judgment are sustained. The physician must realize that above all the patient and family want an expert physician, not a pal or buddy.

When the cancer becomes resistant to therapy and death is imminent, the patient and family need support more than ever to help them through the last days. The family must understand that no known effective therapy remains and that the goal of management must change from destroying cancer cells to providing comfort. Once this is decided, chemotherapy, transfusions, antibiotics, blood counts, and other laboratory tests are no longer necessary. The patient needs to be hospitalized only if proper supportive care or pain medication cannot be given at home. For pain that cannot be controlled by oral analgesics, parenteral morphine is the drug of choice and is most effective when given by continuous intravenous infusion. The inadequate control of cancer pain in the United States is a national scandal. The demonstrably unwarranted fear of narcotic addiction, the rigid adherence to timed dosages irrespective of need, and the lack of knowledge and plain human sensitivity of doctors and nurses are widespread and indefensible. There is no reason for any cancer patient to suffer severe unremitting pain, a consequence of cancer more feared than death by most patients. Very effective narcotic regimens, including self-regulated intravenous drips, are both safe and readily available.

Patients themselves seldom ask the physician at this time whether they are going to die, probably because they already know or suspect the truth and do not want to confront the physician with an uncomfortable question. Should the question be asked, however, the patient probably knows the answer already; to deny this is worse than useless. Although guidelines can be provided for caring for patients during this difficult period, the medical staff must adopt an approach that is suitable to the particular patient and circumstances. Most of all, the patient needs palpable demonstration that the medical staff is readily available and willing to listen, to comfort, to provide any possible service, and simply to be there. Even patients who are at home should not be abandoned; telephone communication can provide welcome support to the family. Both hospice care and home visits by nurses can be a godsend to patients and their families.

155 CANCER PREVENTION

Gilbert S. Omenn

Cancers are diagnosed in 1.2 million people in the United States and claim over 500,000 lives each year, one fourth of all deaths. Fear of cancer, suffering from cancers and their treatment, and the limited benefit of treatments for most common cancers combine to make prevention an increasing priority in clinical medicine and in public health.

As Figure 155–1 shows, the leading cancer killer by far in both men and women is lung cancer, followed by cancers of the prostate, colon and rectum, and pancreas in men and by cancers of the breast, colon and rectum, ovary, and pancreas in women. Pancreatic and pulmonary cancers are particularly lethal.

The primary modalities for cancer prevention (see Table 155–1) involve behavior change, including smoking, alcohol, diet, and physical activity. Reduction of exposures to carcinogenic agents from all environmental sources comes next. Under intensive investigation are hormonal, nutritional, and pharmacologic interventions and genetic screening, counseling, and eventual treatments for those with testable inherited predispositions.

HEALTH-PROMOTING/CANCER-PREVENTING BEHAVIOR CHANGES

SMOKING CESSATION AND SMOKING PREVENTION. Diseases related to cigarette smoking represent a twentieth-century epidemic, now spreading globally. Smoking is the primary cause of cancers of the lung, larynx, oral cavity, and esophagus (approximately 10 to 20 times the risk compared with nonsmokers) and contributory to cancers of the pancreas, bladder, kidney, stomach, and cervix and to leukemia (about 2 times the risk). Smoking acts synergistically with chemical and radiation carcinogens in the lung and with alcohol in the esophagus and oral cavity. Former smokers, after a lag of up to 4 years, show a progressively lower relative risk compared with continuing smokers and even compared with the slowly rising rate as never-smokers age. However, the absolute risk of lung cancer probably never declines, in sharp contrast with coronary heart disease endpoints. Low-tar, low-nicotine, and filtered cigarettes have had little or no protective effect, because the smokers tend to inhale more deeply and more frequently.

Snuff dipping and smokeless tobacco have been promoted successfully to adolescents in recent years; their predisposition to cancer is similar to that of inhaled smoking. Leukoplakia, a white patch involving the oral mucosa epithelium, is a telltale premalignant lesion found in up to half of tobacco chewers, with a 5% risk

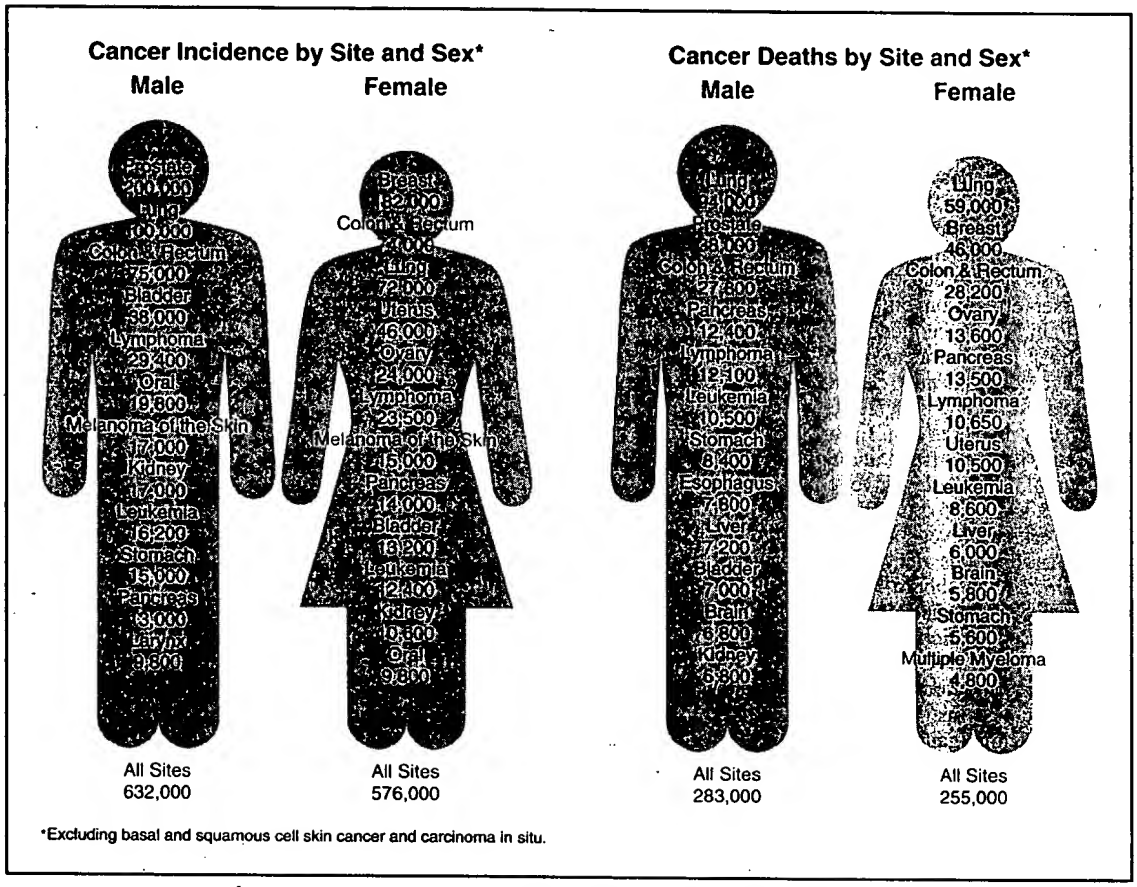

FIGURE 155–1. Leading sites of cancer incidence and death—1994 estimates. (From Cancer Facts and Figures—1994. Atlanta, American Cancer Society, 1994.)

of epidermoid carcinoma. Finally, environmental tobacco smoke (ETS), or second-hand smoke, has been declared a definite human carcinogen by the Environmental Protection Agency; 6000 cases of lung cancer per year are attributed to ETS by the National Research Council.

A huge literature attests to the difficulty of helping smokers quit. About 5% "quit" by themselves (for at least a 6-month period) each year, but others relapse. Physicians play a key role in urging smokers to quit and in guiding them to self-help materials, classes, or pharmacologic quitting aids. Work-site, family, and community reinforcement is essential; increased taxes on tobacco products reinforce as well. Prevention of smoking, especially in young people, minorities, and women, can be enhanced by organized community and school programs as well as regulatory actions.

MODERATION OF ALCOHOL INTAKE. The National Cancer Institute *Dietary Guidelines* recommend that consumption of alcoholic beverages, if any, should be moderate. Alcohol intake is highly associated with cancers of the esophagus, oral cavity, pharynx, and larynx and, less strikingly, with liver, rectal, pancreatic, and breast cancer. It acts synergistically with cigarette smoking.

DIET. Guidelines for healthy diets strongly recommend decreases in fat and increases in fiber intake, most easily described as "five-a-day" fruits and vegetable portions. Such advice aims at preventing cancers, heart disease, and bowel disorders too.

The typical U.S. diet has 39% of calories from fat or about 150 grams per day. Dietary fat intake correlates positively with incidence and mortality rates for breast, prostate, and colon cancers. International, migrant, and time-trend data indicate that reduction in dietary fat to 20% of caloric intake would reduce breast cancer risk by two thirds. Unfortunately, most case-control (retrospective) and cohort (prospective) epidemiologic studies have found less striking correlations or none at all. Similar inconsistencies underlie positive associations of fat intake with colorectal and prostate cancers. Fat intake involves many variables, including percentage of calories, grams per day, saturated versus unsaturated fats and fatty acids, overweight, and duration of diet. Each type of cancer possesses other confounding or interacting risk factors. Experimental studies in rodents show that dietary fat may exert tumor-enhancing or -promoting effects on the breast directly through changes in cell membranes or indirectly through neuroendocrine systems. In the colon, fat may influence bile acids, sterol substrates, and fecal microflora.

Clinical trials are essential to test hypothesized mechanisms and behavior change for cancer prevention. A feasibility study for the Women's Health Trial showed that women aged 45 to 69 can lower mean dietary fat intake to below 25% of energy requirements and maintain the diet and good health for 2 years. Reduction in dietary fat intake is a major component of the Women's Health Initiative, a massive trial aimed at reducing breast cancer, heart disease, and osteoporosis in postmenopausal women.

INCREASE IN DIETARY FIBER. The surgeon Dennis Burkitt deduced from widely varying country rates for colon cancer that

TABLE 155–1. PROPORTIONS OF CANCER DEATHS ATTRIBUTED TO VARIOUS RISK FACTORS

Factor or Class of Factor	Best Estimate	Range of Estimates
Tobacco	30	25–40
Alcohol	3	2–4
Diet	35	10–70
Food additives*	< 1	−5–2
Reproductive/sexual behavior	7	1–13
Occupation	4	2–8
General pollution	2	1–5
Industrial products	< 1	< 1–2
Medicines/medical procedures	1	0.5–3
Geophysical factors†	3	2–4
Infections	10?	1–?

* Minus indicates potential benefits from antioxidants and other additives.
† UV and cosmic radiation included; perhaps 1% truly avoidable.
From Doll R, Peto R: The causes of cancer: Quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 66:1193, 1981.

fiber-rich diets play a protective role. The highest rates occur in western countries with a high intake of refined carbohydrates compared with the naturally occurring fiber-rich foods common in African and Asian countries, where colon cancer rates are low. Low colon cancer rates with a mean intake of 31 grams of fiber per day in Finland contrast with high rates in Denmark and New York on 17 grams of fiber per day despite similar fat intakes. Fiber describes a heterogeneous category, defined by plant origins and resistance to digestion by human enzymes, making measurement awkward. Soluble fibers (gums, mucilages, pectins, and hemicelluloses) delay gastric emptying, slow glucose absorption, and lower serum cholesterol, with lesser effects on bulk and transit time. Insoluble fibers (cellulose, lignin, other hemicelluloses) increase fecal bulk and decrease intestinal transit time. Whole-grain breads, cereals, fruits, vegetables, legumes, and nuts contain lots of fiber but provide fibers of markedly different natures. Cellulose and hemicellulose are found primarily in cereals and grains; lignin, primarily in berry fruits; and pectin, in citrus fruits and apples.

Dozens of epidemiologic studies give consistent findings of a moderate-to-strong protective effect of fiber against colon cancer, as well as a protective effect of vegetables. When one analyzes the different forms of fiber or foods rich in fiber, however, more variable results are obtained. One should remember that no effect of a dietary component can be identified unless there is sufficient variation in intake within the population studied.

INCREASED PHYSICAL ACTIVITY. Overcoming sedentary or inactive lifestyles benefits cardiovascular, respiratory, muscular, cognitive, and metabolic systems. Increased physical activity seems to offer significant protection against colon cancer.

REDUCTION IN EXPOSURES TO ENVIRONMENTAL CARCINOGENIC CHEMICALS. Asbestos fibers, inorganic arsenic compounds, bis-chloromethyl ether, chromium compounds, mustard gas, nickel dusts, and polycyclic aromatic hydrocarbons from coal and gasoline combustion are lung carcinogens; vinyl chloride causes a distinctive angiosarcoma of the liver; some pesticides are associated with the development of non-Hodgkin's lymphoma; aromatic amine dyestuffs can cause bladder cancer; leather production and isopropyl alcohol manufacturing are associated with nasal cancers; and benzene can cause acute myelocytic leukemia. Tobacco smoke is the most prevalent chemical carcinogen, possibly followed by charbroiling of meats and fish.

PHYSICAL AGENTS. Ultraviolet radiation is the primary cause of skin cancers, including melanoma and lip cancer. Ionizing radiation (including radiotherapy) increases rates at essentially all exposed sites. Nonionizing radiation and electromagnetic fields have been suspected of increasing leukemia and brain cancer and possibly breast cancer rates, but the data are not consistent and the relationship is far from demonstrated.

DRUGS. Alkylating agents can cause leukemias; androgen anabolic steroids, liver cancer; chlornaphazine, bladder cancer; estrogens (possibly also "environmental estrogens"), cancers of the vagina and cervix (diethylstilbestrol), endometrium (postmenopausal estrogens), or liver and cervix (steroid contraceptives); azathioprine and cyclosporine immunosuppressants, non-Hodgkin's lymphoma; and phenacetin-containing analgesics, renal pelvic tumors.

INFECTIOUS AGENTS. Specific infectious agents can cause several cancers: primary hepatocellular cancer is associated, with hepatitis B and C (with distinctive mutations in gene *p53* and with synergistic effects of aflatoxins derived from *Aspergillus flavus* growth on crops); cervix, with certain human papillomaviruses; Burkitt's lymphoma and nasopharyngeal, with Epstein-Barr virus; Kaposi's sarcoma and non-Hodgkin's lymphoma, with HIV-1; T-cell leukemia, with HTLV-I; urinary bladder *(Schistosoma haematobium)* and cholangiocarcinoma of the liver *(Clonorchis sinensis),* with parasites; and gastric cancer, with *Helicobacter pylori.* Environmental or antibiotic control of these infections and/or vaccines to protect against exposure can be effective. Population-wide neonatal hepatitis B virus immunization is expected to reduce or eliminate the scourge of primary liver cancer in Taiwan.

CANCER PREVENTION INTERVENTIONS

CHEMOPREVENTION. Population trials of chemopreventives are currently under way worldwide. Because of their apparent antioxidant, tumor suppressor, and immunomodulatory actions, micronutrients (especially carotenoids and retinoids) have been prime agents, based on observational epidemiologic work as well as animal and cell culture findings showing protective effects. Other antioxidants (vitamins E and C and selenium), anticarcinogens in soybeans (protease inhibitors, isoflavones, and phytosterols), and inhibitors of cellular proliferation or tumor promotion are in phase I and phase II studies. For example, calcium supplementation and possibly aspirin and other nonsteroidal anti-inflammatory agents can reduce colonic cell proliferation in humans.

The largest current studies involve beta-carotene alone (22,000 male physicians), beta-carotene plus vitamin E (29,000 male smokers in Finland), beta-carotene plus vitamin A (14,000 male and female U.S. smokers and 4000 asbestos-exposed workers), and beta-carotene plus vitamin E plus aspirin (40,000 female health professionals). The first large trial to report its findings shocked the medical and vitamin supplement worlds. In April of 1994, the Alpha-Tocopherol/Beta-Carotene study in Finland reported not only no benefit from vitamin E or from beta-carotene but also 18% lung cancer and 8% overall mortality rate increases (both statistically significant) in the men receiving beta-carotene, 20 mg per day. This unexpected and unexplained result firmly demonstrates that seemingly logical approaches must be tested in randomized, clinical preventive trials before their merits are accepted. We await results from the other trials.

HORMONES. Cancers of the hormone-responsive tissues account for 20% of male and more than 40% of female newly diagnosed cancers in the United States. Thus chemoprevention with "antihormones" represents a promising approach. Progesterone is the prototype. Oral contraceptives (OC's) have become potent cancer prevention agents, once the early sequential OC's (which increased endometrial cancer risk) were replaced with estrogen-progesterone combinations. Women with 6 or more years of OC use have less than one-sixth the risk of endometrial cancer compared with never-users, and the effect lasts at least 15 years after discontinuation of the OC's. Combination OC's also suppress gonadotropin levels and ovulation, thereby decreasing the risk for epithelial ovarian cancers by about 40%, independent of parity. The breast is different: Progesterone increases the rate of cell division beyond that induced by estrogen. Combination therapy is now also preferred for postmenopausal hormone replacement therapy.

A strategy in premenopausal women for gaining the benefits of the OC's while actually reducing breast cancer (and cardiovascular) risk involves use of luteinizing hormone–releasing hormone (LHRH) antagonists, a "reversible bilateral oophorectomy," plus low-dose estrogen to overcome hypoestrogenic effects plus a quarterly progestogen. Antiestrogenic agents, such as tamoxifen, also are being subjected to multiple-endpoint trials.

Diethylstilbestrol and LHRH agonists are effective therapeutically against metastatic prostate cancer by reducing testosterone-mediated maintenance of prostate tissue. Inhibitors of 5-α-reductase may become useful in high-risk patients or even in primary prevention.

GENETIC SCREENING. Molecular studies in cancer reveal numerous oncogenes, tumor-suppressor genes, genes affecting cell division, cell cycle, and cell proliferation, and a host of other potential targets for cancer prevention. Known inherited cancer syndromes, such as retinoblastoma and polyposis coli, have specific mutations of general interest in carcinogenesis. If and when highly predisposing breast cancer gene(s) and cell-cycle/tumor-suppressor genes (such as the already discovered multiple-tumor-suppressor [MTS-1] p16 mutant) can be identified and converted into diagnostic tests, genetic screening and counseling programs are bound to increase. The discovery of other genetic mechanisms will bring new types of interventions as well.

American Cancer Society: Cancer Facts and Figures—1994. Atlanta, American Cancer Society, 1994. *Excellent annual update on cancer statistics and advances.*

DeVita VT Jr, Hellman S, Rosenberg SA (eds.): Cancer, Principles and Practice of Oncology. 4th ed. Philadelphia, JB Lippincott, 1993. *See Chapters 9 (Causes of Cancer) and 20 (Cancer Prevention).*

Doll R, Peto R: The causes of cancer: Quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 66:1193, 1981. *Now-classic analysis of the preventable causes of cancer mortality.*

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group: The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. N Engl J Med 330:1029, 1994. *The first large-scale antioxidant chemoprevention trial reported unexpected and unexplained results.*

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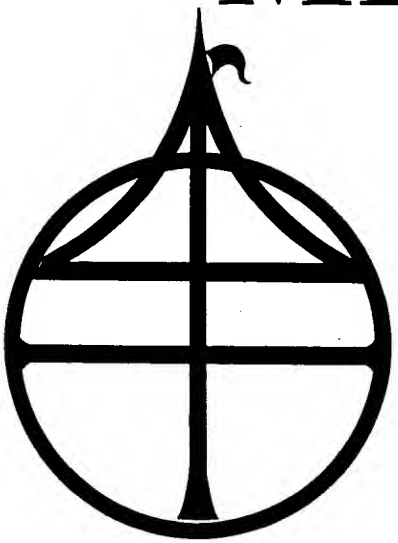

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TABLE 412–1. NEUROLEPTIC-INDUCED MOVEMENT DISORDERS

Acute Disorders	Chronic Disorders
Dystonic reaction	Tardive dyskinesia
Parkinsonism	Stereotypic: oral-facial-lingual-masticatory
Akathisia	Trunk-pelvic
Neuroleptic malignant syndrome	Respiratory
	Choreic: limbs
	Tardive dystonia; tics; myoclonus; tremor; akathisia; parkinsonism

frequency and severity of tics and ameliorate impulsive and aggressive behavior. These drugs, however, cause sedation, depression, and weight gain. Furthermore, tardive dyskinesia is a potentially serious complication of chronic neuroleptic therapy. Clonazepam, clonidine, fluoxetine, and clomipramine seem to be particularly helpful in the treatment of obsessive-compulsive disorder and other behavioral problems frequently associated with Tourette's syndrome.

MYOCLONUS

Myoclonus is a jerklike movement produced by a sudden, rapid, and brief contraction (positive myoclonus) or a muscle inhibition (negative myoclonus). *Segmental myoclonus* usually involves either the branchial structures, innervated by the lower cranial nerves and upper cervical nerve roots, or other body parts innervated by the spinal roots and nerves; it consists of rhythmic (1 to 3 Hz) contractions caused by a lesion of the brain stem or spinal cord. *Palatal myoclonus* results from acute or chronic lesions involving the anatomic triangle linking dentate, red, and inferior olivary nuclei. *Generalized myoclonus* is believed to reflect discharges arising from the brain stem reticular formation and is categorized as physiologic, essential, epileptic, or symptomatic. Two forms of myoclonus are associated with sleep: physiologic sleep myoclonus, occurring normally during initial phases of sleep, and nocturnal myoclonus, now called *"periodic movements of sleep,"* often associated with *"restless legs syndrome"* as well as with abnormal involuntary movements while the person is awake.

Causes of generalized myoclonus include acute and prolonged hypoxia and ischemia; various metabolic, infectious, and toxic factors; and exposure to neuroleptic drugs (tardive myoclonus). Myoclonus can be associated with familial chorea and dystonia and with many neurodegenerative disorders, including parkinsonism, progressive myoclonus epilepsy, and a variety of rare heredodegenerative disorders. Multifocal myoclonus often develops in the late stages of Creutzfeldt-Jakob disease and, less frequently, Alzheimer's disease.

The specific pathogeneses of myoclonus are unknown. Clonazepam, lorazepam, valproate, carbamazepine, and 5-hydroxytryptophan have been reported to have antimyoclonic activity. Clonazepam, at a dosage of 1 to 9 mg per day, is the drug of first choice, but the development of adverse effects, such as drowsiness, ataxia, and sexual dysfunction, often limits its usefulness.

STEREOTYPIES

The term "stereotypy" denotes a continuous or intermittent involuntary, coordinated, patterned, repetitive, rhythmic, purposeless, but seemingly purposeful and ritualistic movement. Stereotypies may be simple (e.g., chewing movement, foot tapping, body rocking) or complex (e.g., complicated rituals, sitting down and arising from a chair). They can be volitionally suppressed. Stereotypies can accompany a variety of human behavioral disorders, such as anxiety, obsessive-compulsive disorders, Tourette's syndrome, schizophrenia, akathisia, autism, and mental retardation. Stereotypies and self-stimulatory or self-injurious behavior constitute the most recognizable symptoms in mentally retarded and autistic patients.

Tardive dyskinesia, a persistent movement disorder caused by exposure to dopamine receptor blocking drugs, is a frequently encountered stereotypy. Many other tardive movement disorders can result from the use of dopamine receptor blocking drugs (neuroleptics) (Table 412–1). The term "akathisia" describes the combination of stereotypy and a sensory component, such as an inner feeling of restlessness. The disorder particularly affects the lower extremities ("restless legs") and often is worse at night, causing insomnia, and it may be associated with periodic movements of sleep (see Ch 397). Elderly women appear to be at particularly high risk for tardive dyskinesia. The mechanism of the disorder is poorly understood but is believed to result from the development of supersensitive dopamine receptors caused by chronic neuroleptic blockade. Prevention is the best treatment for the drug-induced movement disorders. Whenever possible, drugs other than the neuroleptics should be used for psychiatric or gastrointestinal problems. When no alternative exists, the dosage and duration of exposure should be kept at a minimum. Spontaneous remissions of tardive dyskinesia occasionally follow withdrawal of the offending agent. Dopamine-depleting drugs, such as tetrabenazine or reserpine, are the most effective drugs in its symptomatic treatment.

Jankovic J: Tardive myoclonus and other drug-induced movement diseases. Clin Neuropharmacol, in press. *A comprehensive review of clinical and pharmacological features of tardive dyskinesias and other movement disorders produced by dopaminergic or antidopaminergic drugs.*

Jankovic J: Tourette's syndrome: Phenomenology, pathophysiology, genetics, epidemiology and treatment. *In* Appel SH (ed.): Current Neurology, Vol 13. Chicago, Mosby-Year Book, 1993, p 209. *A critical review of current knowledge about the motor and behavioral aspects of Tourette's syndrome.*

Marsden CD, Fahn S (eds.): Movement Disorders 3. London, Butterworth-Heinemann, 1994, p 503. *A comprehensive review of movement disorders, including tics, myoclonus, and stereotypies.*

Section Five— Degenerative Diseases of the Nervous System

Robert B. Layzer

The term "degenerative diseases" refers to a varied assortment of central nervous system disorders characterized by gradual and progressive loss of neural tissue. This section deals with several degenerative diseases of unknown cause: the hereditary ataxias, paraplegias, and amyotrophies; the phakomatoses; syringomyelia; and amyotrophic lateral sclerosis. Several important diseases are discussed in other chapters concerned with dementia, extrapyramidal diseases, and autonomic disorders. Some degenerative diseases are difficult to classify because they involve multiple anatomic locations; these *multisystem atrophies* have arbitrarily been assigned to the chapters that deal with their principal symptom (see Table 413–1).

413 HEREDITARY CEREBELLAR ATAXIAS AND RELATED DISORDERS

The symptoms of hereditary ataxia may be intermittent or progressive. *Intermittent or periodic ataxia* occurs in children with a variety of recessively inherited biochemical disorders, such as aminoacidurias and disorders of pyruvate metabolism. A rare, autosomal dominant disease known as hereditary periodic ataxia is characterized by attacks of vertigo, nystagmus, ataxia, and dysarthria, lasting several hours; it responds to prophylactic treatment with acetazolamide.

Progressive ataxia occurs in children with known biochemical disorders such as abetalipoproteinemia and some of the lipidoses, but most diseases in this category are of unknown origin. Those that begin before age 20, including Friedreich's ataxia and ataxia-telangiectasia, are usually inherited in an autosomal recessive fashion, whereas most adult-onset types are autosomal dominant.

FRIEDREICH'S ATAXIA

This autosomal recessive disease, with a carrier frequency of nearly 1 in 100 and a prevalence of 2 in 100,000, is probably the most common type of hereditary ataxia. The biochemical mechanism is unknown, but the abnormal gene has been mapped to the long arm of chromosome 9.

PATHOLOGY. At autopsy the spinal cord is atrophic. There is loss of nerve cells in the dorsal root ganglia and Clarke's columns and "dying-back" degeneration of nerve fibers in the dorsal columns, pyramidal tracts, spinocerebellar tracts, and peripheral nerves. Minor changes are present in the brain stem and cerebellum. The heart shows chronic interstitial fibrosis and ventricular hypertrophy.

CLINICAL MANIFESTATIONS. Progressive ataxia of gait usually begins in childhood or adolescence and within a few years is accompanied by loss of deep reflexes, limb ataxia, Babinski signs, and cerebellar dysarthria. The ability to walk is lost about 15 years after onset. Most patients eventually exhibit scoliosis, pronounced impairment of vibration and position sense in the lower extremities, and pes cavus. Some develop wasting of distal limb muscles, a stocking-glove deficit of superficial sensation, nystagmus, deafness, or optic atrophy. Intellect remains normal. A hypertrophic cardiomyopathy is present in most patients and often leads to supraventricular arrhythmias; heart failure is probably the major cause of death. Insulin-dependent diabetes mellitus develops in 10 to 20% of patients. The mean age at death is 37 years.

DIAGNOSIS. Sensory nerve action potentials are small or absent. Electromyography may show signs of denervation in distal limb muscles, but motor nerve conduction velocities are normal. The cerebrospinal fluid is normal except for mild elevation of the protein content in a few cases. Magnetic resonance (MR) imaging often shows atrophy of the cervical spinal cord; the medulla may also be small, but the cerebellum is spared. Electrocardiography often shows inverted T waves, right- or left-axis deviation, and right or left ventricular hypertrophy; conduction disturbances are uncommon.

DIFFERENTIAL DIAGNOSIS. The constellation of progressive ataxia, areflexia, Babinski signs, and onset before age 25 is usually diagnostic. However, a similar picture can occur in vitamin B_{12} deficiency and in two hereditary autosomal recessive disorders, abetalipoproteinemia and selective vitamin E deficiency. True Friedreich's ataxia is sometimes confused with a less common autosomal recessive type of early-onset progressive ataxia, in which the tendon reflexes are preserved; in the latter syndrome, optic atrophy, scoliosis, and electrocardiographic abnormalities are rare.

ATAXIA-TELANGIECTASIA

Ataxia-telangiectasia is an autosomal recessive, multisystem disease affecting the skin, nervous system, and immune system. Its prevalence has been estimated at 1 to 2 per 100,000. The condition is described further in Ch. 223.

ADULT-ONSET CEREBELLAR ATAXIA

Hereditary ataxia starting in adult life is nearly always an autosomal dominant disorder with multiple neurologic manifestations, among which cerebellar signs are prominent. In Europe the prevalence is 1 to 10 per 100,000 population. The syndrome is genetically heterogeneous; one type is caused by expansion of an unstable trinucleotide repeat on chromosome 6p, another is linked to chromosome 12q, and the Azorean form (Machado-Joseph disease) is linked to chromosome 14q.

PATHOLOGY. Many cases have the pathologic features of olivopontocerebellar atrophy, with loss of neurons in the inferior olives and pontine nuclei (which provide major afferent pathways to the cerebellum), as well as degeneration of the spinocerebellar tracts, corticospinal tracts, and posterior columns. Neuronal degeneration is sometimes found in the cerebellar cortex, dentate nucleus, basal ganglia, midbrain, cerebral cortex, and spinal cord, including the anterior horns. The pathology, however, is as variable as the clinical findings, even within a given family. In Machado-Joseph disease, the cerebellar cortex and olives are spared.

CLINICAL MANIFESTATIONS. The age of onset, although variable, is usually between 20 and 50. Cerebellar ataxia of gait, dysarthria, and incoordination of the limbs usually dominate the clinical picture, so that the ability to walk is lost within 15 years. The other manifestations are extremely variable. Babinski signs and increased reflexes are commonly present, and some patients have spastic weakness in the legs. Vibration and position sense are sometimes lost as the disease advances, and the reflexes may disappear as the primary sensory neurons degenerate. Extrapyramidal findings may include impassive facies, cogwheel rigidity, chorea, athetosis, dystonia, and facial dyskinesia. Many patients have supranuclear oculomotor disorders such as lid retraction, ptosis, nystagmus, slow eye movements, and gaze paresis, especially upgaze. Optic atrophy, with pale discs, is common. Pigmentary degeneration of the retina, beginning in the macula, is an early and constant feature in some families, suggesting that these cases may be genetically distinct. Personality change or dementia, muscle wasting and fasciculation in the tongue and distal extremities, and bulbar symptoms of dysphagia or hoarseness are other common manifestations. Death occurs approximately 20 years after onset, at an average age of 57.

TABLE 413-1. THE MULTISYSTEM ATROPHIES

Disease	Heredity	Principal Feature	Associated Features	Chapter
Shy-Drager syndrome	Sporadic	Autonomic insufficiency	Parkinsonism, cerebellar ataxia, dysphagia, laryngeal stridor, amyotrophy	402
Progressive supranuclear palsy	Sporadic	Ophthalmoplegia, especially vertical	Gait ataxia, axial dystonia, parkinsonism, pseudobulbar palsy, dementia	410
Kearns-Sayre syndrome	Sporadic	Ptosis and ophthalmoplegia	Short stature, cerebellar ataxia, retinal degeneration, heart block, deafness, mitochondrial myopathy, mental deficiency, Babinski signs	454
Hereditary ataxias, adult type	Autosomal dominant	Cerebellar ataxia	Ophthalmoplegia, dementia, parkinsonism, dystonia, optic atrophy, retinal degeneration, dysphagia, amyotrophy	413

A few families seem to have a "pure" cerebellar syndrome beginning in the seventh decade of life. These cases (cerebellar cortical atrophy) tend to have a more benign prognosis.

DIAGNOSIS. In the olivopontocerebellar atrophy syndrome, MRI may show atrophy of the cerebellar folia and pons, with enlargement of the fourth ventricle and pontine cisterns. In pure cerebellar cortical atrophy, only the cerebellum is shrunken. The cerebrospinal fluid is usually normal. Sensory nerve action potentials are small or absent in patients with absent reflexes; in patients with preserved reflexes, somatosensory evoked potentials may be abnormal.

DIFFERENTIAL DIAGNOSIS. Nonhereditary cases of late-onset cerebellar degeneration are at least as common as the hereditary kind. Some are associated with alcoholism or a visceral malignancy, but in many, no apparent cause can be established. These patients' cerebellar symptoms tend to begin between the ages of 40 and 60 and may be accompanied by dementia, extrapyramidal signs, or Babinski signs. Some cases of this kind have the pathologic features of olivopontocerebellar atrophy, but whether there is any genetic link to the autosomal dominant ataxias is unclear. It should be noted that patients presenting with ataxia may later develop the typical signs of progressive supranuclear palsy or one of the other multisystem atrophies listed in Table 413–1.

Harding AE: The Hereditary Ataxias and Related Disorders. Edinburgh. Churchill Livingstone, 1984. *A detailed review of the hereditary cerebellar ataxias and spastic paraplegias, including the author's own study of several hundred patients and family members. A modern classic.*

Orr HT, Chung M, Banfi S, et al.: Expansion of an unstable trinucleotide repeat in spinocerebellar ataxia type 1. Nature Genetics 4:221, 1993. *Reports the first specific gene defect to be found in a hereditary ataxia.*

414 HEREDITARY SPASTIC PARAPLEGIAS

This is a diverse group of uncommon diseases whose main symptom is an insidiously beginning, progressive spasticity of the lower extremities. Families with "pure" hereditary spastic paraplegia (Strümpell's disease) are the most numerous, but many rare variants have been reported in which spasticity is associated with other neurologic, ocular, or cutaneous manifestations, overlapping with the spinocerebellar degenerations. The prevalence of these diseases is not well established. Rare examples of *primary lateral sclerosis,* although sporadic in incidence, may belong to this class.

PATHOLOGY. In the pure form, the spinal cord shows degeneration of the lateral corticospinal tracts and posterior columns, most severe in the thoracic region. Less often there is minor degeneration of the spinocerebellar tracts, anterior corticospinal tracts, anterior horn cells, and cortical Betz cells.

CLINICAL MANIFESTATIONS. Most patients with pure hereditary spastic paraplegia continue to walk for many years and have a normal lifespan. Many cases begin in infancy with delayed walking, but the onset can be as late as the seventh decade. Spasticity of the legs and a stiff, slow gait are the main symptoms. Affected persons walk on their toes, trip easily, and are unable to run. About one fourth have pes cavus. The legs show hyperactive reflexes, clonus, and Babinski signs, whereas the arms are usually normal. Later the legs may become weak, the arms may show increased reflexes, and distal muscle wasting may develop, especially in the hands. Vibration and position sense may become impaired in the legs, and many patients develop urinary frequency, urgency, and precipitancy, although sexual function remains normal. Most patients become unable to walk sometime in the sixth or seventh decade.

DIAGNOSIS. The cerebrospinal fluid is normal. Electromyography may show denervation in the distal limb muscles, but the sensory nerve action potentials are preserved, even in patients showing decreased vibration and position sense. Somatosensory evoked potentials, however, are consistently small or unobtainable, reflecting a degeneration of dorsal column fibers.

DIFFERENTIAL DIAGNOSIS. Hereditary spastic paraplegia must be distinguished from nonhereditary causes of slowly progressive myelopathy such as cervical spondylosis, intraspinal tumor, arteriovenous malformation or fistula of the spinal cord, multiple sclerosis, amyotrophic lateral sclerosis, and myelopathy associated with human T cell lymphotropic virus 1 (HTLV-1 tropical spastic paraparesis, Ch. 428.3). MRI has simplified diagnosis of many of these conditions.

415 HEREDITARY AND ACQUIRED INTRINSIC MOTOR NEURON DISEASES

Degenerative diseases of several kinds can attack the large motor neurons of the spinal cord or the brain to produce selective impairment of muscle strength or motor skill. Those of childhood are largely hereditary, whereas the major adult disorder, amyotrophic lateral sclerosis, is nearly always sporadic, with few clues illuminating either its cause or molecular pathogenesis. Table 415–1 lists the major disorders in this category, and the references provide greater detail on the many subtypes.

HEREDITARY AMYOTROPHIES

Hereditary spinal muscular atrophy is a syndrome of progressive muscular weakness and atrophy resulting from selective degeneration of the motor neurons of the spinal cord. A comparable disorder of the lower brain stem nuclei produces progressive bulbar palsy. Many different clinical syndromes have been delineated based on the age of onset, the pattern of muscular weakness, the rate of progression, and the mode of inheritance. Using this approach, at least 15 separate genetic disorders can be recognized. It has been estimated that 1 in 40 Caucasians carries a gene for spinal muscular atrophy. No consistent biochemical defect is known, although hexosaminidase deficiency has been identified in a few cases.

PATHOLOGY. At the time of postmortem examination in the spinal cases, the anterior horns show gliosis and loss of large neurons, and many of the remaining motor neurons are undergoing degeneration. The ventral roots are atrophic owing to loss of myelinated nerve fibers. Similar changes are observed in the motor nuclei of the brain stem in bulbar cases.

In the well-developed infantile and childhood types, microscopic examination of the skeletal muscles using histochemical techniques shows large groups of round, atrophic muscle fibers and large groups of hypertrophied fibers staining uniformly as either type 1 or type 2. These features reflect the continuing process of denervation and reinnervation. However, at an early stage of infantile spinal muscular atrophy the only finding may be uniform atrophy of all

TABLE 415–1. THE MAJOR INTRINSIC MOTOR NEURON DISEASES

Hereditary
Spinal muscular atrophy
Type I. Acute, infantile (Werdnig-Hoffmann disease)
Type II. Late infantile and childhood type
Type III. Juvenile and adult types
Familial amyotrophic lateral sclerosis (ALS)
Acquired
Acute: anterior poliomyelitis
Chronic:
ALS alone
Anterior horn cell degeneration associated with spinocerebellar degeneration, Shy-Drager syndrome, parkinsonism, Creutzfeldt-Jakob disease
Remote neoplasms, other
Primary lateral sclerosis (rare)

muscle fibers, with preservation of the normal "checkerboard" fiber-type pattern. In slowly progressive cases of juvenile or adult onset, atrophic muscle fibers are found mainly in small groups; most muscle fibers are of normal size but are arranged in groups of uniform fiber type. After many years some muscle fibers show secondary myopathic changes, such as internal nuclei, splitting, or degeneration.

ACUTE INFANTILE SPINAL MUSCULAR ATROPHY

Werdnig-Hoffmann disease is a fatal, early infantile form of spinal and bulbar muscular atrophy that appears to be a single genetic entity. Inherited as an autosomal recessive abnormality on chromosome 5q, it is one of the most common fatal hereditary diseases of childhood, with an annual incidence of 1 in 20,000 live births and a carrier frequency in the general population of about 1 in 80. The abnormal gene is located on the long arm of chromosome 5.

In at least one third of the cases, there is a prenatal onset, with reduced fetal movements, weakness at birth, or congenital joint deformities. In the remainder of cases, the disease becomes apparent in the first 2 or 3 months of life. There is progressive, flaccid weakness of the trunk and limbs, with severe hypotonia, poor head control, and diminished movements of the limbs, more severe in the proximal muscles. Weakness of the intercostal muscles causes retraction of the chest during inspiration; the cry is weak, and coughing is ineffective. Bulbar weakness causes difficulty in sucking and swallowing. The tendon reflexes are usually absent. Death occurs before 3 years of age; 50% of the patients die in the first 7 months of life and 95% in the first 18 months.

The serum creatine kinase activity and the cerebrospinal fluid are normal. Electromyography shows reduced activation of motor unit potentials, many of which are of increased size, duration, and complexity. Fibrillations and fasciculations are rarely observed. It is important to distinguish this disease from treatable disorders such as infant botulism and chronic inflammatory polyneuropathy. The former is identified by repetitive nerve stimulation tests showing abnormal neuromuscular transmission and the latter, by abnormalities of nerve conduction and increased protein levels in the cerebrospinal fluid.

PROGRESSIVE MUSCULAR ATROPHY IN CHILDREN

PROXIMAL TYPE. Clinically, this is a rather diverse disorder, but most cases are now thought to be caused by a single autosomal recessive gene, located on the long arm of chromosome 5, near or at the locus for Werdnig-Hoffmann disease. The incidence of this syndrome is 1 in 24,000 live births, and the carrier rate is approximately 1 in 90. A milder, autosomal dominant form is also known.

Weakness starts anytime from birth to 8 years of age, usually before 1 year of age. The weakness affects the trunk and limbs and initially is more severe in proximal muscles. The limb muscles become atrophic, the tendon reflexes are lost, and joint contractures may develop. Fasciculations are not prominent but may be apparent in the fingers, producing a fine, irregular tremor. The face and jaws may be weak, and the tongue may be atrophic and show fasciculation.

Children with early onset may never be able to walk and often develop severe scoliosis, limb deformities, and respiratory insufficiency. Many eventually die of pulmonary infection, but some very weak patients survive into adult life, the progress of the disease apparently having arrested early in childhood. Children with a later onset of weakness tend to have a milder course, with slowly progressive proximal weakness, increased lumbar lordosis, and a waddling gait. Those with autosomal recessive inheritance rarely walk after age 20, whereas those with the rare autosomal dominant form may still be walking in middle age.

Serum creatine kinase activity may be mildly or moderately increased in patients with slowly progressive weakness, apparently because of secondary myopathic changes in muscle. The cerebrospinal fluid is normal. Electromyography shows the typical changes of chronic denervation and reinnervation as well as fibrillations and fasciculations, serving to distinguish these patients from similar patients with muscular dystrophy.

Many of these children benefit from active and passive physical therapy and the judicious use of lightweight braces. Special attention should be given to spinal support to counteract scoliosis. Later in childhood, surgical immobilization of the spine may be indicated.

DISTAL TYPE. This category includes both dominant and recessive disorders and accounts for about 10% of all cases of spinal muscular atrophy. Distal limb weakness and muscle wasting, more severe in the lower extremities, usually begins in early childhood and tends to be mild and slowly progressive. Three quarters of the patients have pes cavus, and, except for the absence of sensory deficits, the disorder is often clinically indistinguishable from Charcot-Marie-Tooth disease. However, patients with spinal muscular atrophy have normal conduction in motor and sensory nerves. A rare scapuloperoneal type, with autosomal recessive inheritance, is characterized by distal leg weakness and scapular winging, starting in infancy; there may also be bulbar symptoms such as laryngeal stridor.

SPINAL MUSCULAR ATROPHY OF ADOLESCENT OR ADULT ONSET

Patients with late-onset spinal muscular atrophy have slowly progressive muscular weakness and usually continue to walk for two or three decades or more. Although much less common than the infantile and childhood types, the adult types include at least four clinical and eight genetic categories.

PROXIMAL TYPE. These patients resemble those with muscular dystrophy, and clinical examination may offer few clues to the neurogenic character of the proximal weakness. Fasciculations and muscle cramps are usually not prominent, and the serum creatine kinase activity may be substantially increased. To add to the confusion, males with onset of symptoms in their teens may have large calves. Some patients eventually develop mild bulbar symptoms, such as dysphagia. Electromyography serves to establish the neurogenic nature of the disorder, and muscle biopsy is rarely needed. Families with autosomal dominant and autosomal recessive inheritance have been described. A distinctive X-linked recessive variety, known as bulbospinal neuronopathy, is associated with gynecomastia and dysphagia. It is caused by expansion of a trinucleotide repeat within the gene coding for the androgen receptor.

SCAPULOPERONEAL AND FACIOSCAPULOHUMERAL TYPES. Both myopathic and neurogenic scapuloperoneal syndromes are known, and several varieties begin in the second or third decade of life. Autosomal dominant, autosomal recessive, and X-linked recessive forms have been described. The common feature of these disorders is progressive atrophy and weakness of the shoulder girdle and lower leg muscles, although weakness eventually may spread to the other limb muscles. Electromyography and muscle biopsy can distinguish the anterior horn cell diseases from the muscular dystrophies, but the prognosis is similar in both groups. A few families have an autosomal dominant form of spinal muscular atrophy resembling facioscapulohumeral muscular dystrophy.

DISTAL TYPE. This is usually a childhood disorder, but there are a few families with distal amyotrophy beginning in the third or fourth decade of life, inherited as an autosomal dominant trait. Some familial as well as adult cases exhibit onset in middle age and such a slow progression as never to be incapacitating, even in old age.

AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic lateral sclerosis (ALS) is a fatal degenerative disease of the central nervous system characterized by slowly progressive paralysis of the voluntary muscles.

INCIDENCE. The annual incidence is about 1 case per 100,000 population, the prevalence being 4 to 6 cases per 100,000. Geographical pockets of much higher incidence in Guam, the Kii peninsula of Japan, and western New Guinea suggest possible, still unknown, exogenous causes. Ninety-five percent of cases in the United States are sporadic, but a few families have several members with the typical clinical picture of sporadic ALS arising in an autosomal dominant pattern. Males are affected slightly more often than females. Although the disease can appear as early as the third decade of life, most cases begin after age 40, and the incidence increases into the eighth decade.

PATHOLOGY. Degeneration of the motor neurons of the spinal cord and lower brain stem is marked by extensive cell loss and astrocytic gliosis. Swellings containing neurofilaments are often found on axons close to their cell bodies. As the Betz cells and large pyramidal neurons of the motor cortex disappear, the corticospinal

tracts degenerate, leaving gliosis of the lateral columns of the spinal cord. The ventral spinal roots are depleted of large myelinated nerve fibers, but surviving axons develop distal sprouts that reinnervate some muscle fibers, so that skeletal muscle histopathology shows both muscle fiber atrophy and fiber-type grouping.

ETIOLOGY. Few clues exist to the cause of ALS. Some authors regard the disease as a manifestation of premature aging or a deficiency of a neurotrophic factor. Other speculations include toxic exposure to minerals such as lead or aluminum, deficiency of calcium or magnesium, infection by an unidentified virus, and autoimmunity. Benign paraproteinemia has been encountered in a small proportion of patients, and antiganglioside antibodies have been found in the serum in a majority of the cases, but the significance of these findings is unclear. Recently, some familial cases were linked to a gene coding for the enzyme superoxide dismutase, located on chromosome 21q. This finding has stimulated research into a possible role of free radical toxicity in the pathogenesis of ALS.

CLINICAL MANIFESTATIONS. The major symptom consists of slowly progressive muscle weakness involving the limbs, trunk, breathing muscles, throat, and tongue. Most patients have a mixture of lower and upper motor neuron symptoms, although either may predominate. The former include muscle weakness, wasting, fasciculations, and cramps; the latter include stiffness and slowness of movement, slow and clumsy speech, and explosive release of laughter and crying (pseudobulbar palsy). The ocular muscles are not affected except in patients who survive long times after bulbar paralysis has begun. No impairment affects bladder, bowel, or sexual function. The stretch reflexes are diminished in severely denervated muscles, but more often signs of lower motor neuron weakness are combined with brisk reflexes, a finding nearly specific to ALS. Babinski signs are often present. Sensation is normal except for an expected diminution of vibration sense in the feet in older patients. Occasional cases develop a progressive dementia.

The onset is insidious, and initial symptoms may be confined to a single limb (especially the distal muscles), both limbs on one side, or to lower cranial nerves. Gradually the patchy and asymmetric weakness becomes widespread, and patients become unable to walk, dress, or feed themselves. There is loss of weight because of muscle atrophy and impaired swallowing; the speech becomes unintelligible; choking interferes with eating and sleeping; and breathing becomes difficult even at rest. Death occurs from pulmonary infection and insufficiency. The average survival is 3 years after onset of symptoms, but a few severely debilitated patients live for 10 years or longer.

DIAGNOSIS. Because there are no specific laboratory tests, the diagnosis is based principally on clinical criteria. The disease to be diagnosed as ALS should have a relentlessly progressive, gradual course; lower motor neuron signs should exist at widely separate levels of the nervous system, or upper motor neuron signs should be found well above the level of the lower motor neuron signs; and no conflicting findings such as sensory loss, incontinence, or ocular weakness should be present. The cerebrospinal fluid is normal except for a mild elevation of protein concentration in some cases. Brain and spinal cord imaging is unrevealing. Electromyography shows active and chronic denervation in multiple muscles of the brain stem, upper and lower extremities, and trunk; motor nerve conduction velocity is normal or slightly reduced, and sensory nerve conduction is normal. Serum creatine kinase activity is normal or moderately increased.

DIFFERENTIAL DIAGNOSIS. Although ALS is nearly always fatal, a few patients stop deteriorating or even recover normal strength, but such cases are extremely rare. Other motor neuron disorders, treatable myelopathies and neuropathies, and even thyrotoxic myopathy must be distinguished from ALS (Table 415–2).

TREATMENT. With a disease as grim as ALS, the physician must be careful to avoid premature misdiagnosis. Once the diagnosis is certain, however, some explanation must be given to the patient and the family. This requires considerable tact and gentleness; often it is best to convey the information gradually on successive visits, allowing the relentless progression of weakness to speak for itself.

No medication has been shown to be beneficial, and physical therapy does not delay the neuromuscular deterioration. Quack remedies surface periodically; for their own protection, patients who wish to try experimental forms of treatment should be referred to a reputable academic center.

Patients with impaired gait may benefit from using a cane or walker, and patients who suffer from severe dysphagia without other disabling symptoms can be offered nasogastric tube feeding or a gastrostomy. The most difficult medical question, however, involves the therapeutic role of artificial ventilation. Most patients understanding the hopeless prognosis, prefer not to be kept alive artificially in a state of total paralysis, unable to communicate except with eye movements. Nevertheless, some of these patients have survived for several years, living at home with the help of a devoted and intelligent family. It is important to discuss these issues when patients are in the early stages of respiratory involvement, so that they can make decisions in advance about whether or not to accept emergency resuscitation during a respiratory crisis.

TABLE 415–2. DIFFERENTIAL DIAGNOSIS OF AMYOTROPHIC LATERAL SCLEROSIS

Disease	Distinguishing Features
Benign fasciculations	No weakness, atrophy, or electromyographic (EMG) abnormality
Motor neuron diseases	
*Lead or mercury toxicity	Increased lead or mercury levels
Benign focal amyotrophy	Onset in youth, strictly focal, no upper motor neuron signs
Postpolio progressive muscular atrophy	Slow course, no upper motor neuron signs
Subacute motor neuronopathy in lymphoma	Plateau in few months, later improvement
*ALS in lung cancer or B cell dyscrasia	Improves on treatment of tumor
Hereditary spinal muscular atrophy	Symmetric, slow course, no upper motor neuron signs
*Thyrotoxic myopathy with fasciculations	Myopathic EMG
*Compressive myelopathy due to cervical spondylosis or extramedullary tumor	Sensory symptoms, no lower motor neuron signs in legs, cord compression on MRI or myelography
*Immune-mediated multifocal motor neuropathy	Multifocal nerve conduction block, very high antiganglioside antibody titers

* Treatable conditions

PRIMARY LATERAL SCLEROSIS

Primary lateral sclerosis (PLS) denotes a rare condition characterized by painless, gradually progressing spastic weakness that involves the lower limbs and may ascend to involve the arms and bulbar muscles. In most instances, the disease begins in middle or late life and usually lasts more than a decade before intercurrent illness causes death. Typically, neurologic examinations show a relatively symmetric spastic paraparesis or quadriparesis with heightened deep tendon reflexes and extensor plantar responses but no hint of sensory abnormality. Neither clinical nor electrical studies detect evidence of skeletal muscular denervation. Similarly, imaging procedures disclose no relevant abnormalities involving either brain or spinal cord. The cerebrospinal fluid remains unremarkable, and appropriate tests fail to disclose HIV, HTLV, or other inflammatory processes. Autopsy examinations, performed in a number of cases, have revealed ascending bilateral demyelination of the thoracolumbar corticospinal tracts extending anywhere from the lower cord up to the cerebral peduncles. Cerebral degeneration has not been noted. The cause of PLS is not known, but sporadically arising familial spastic paraplegia cannot be excluded in cases selectively involving the lower extremities. No specific treatment exists, although baclofen may bring modest relief of stiffness.

Brzustowicz LM, Lehner T, Castilla LH, et al.: Genetic mapping of chronic childhood-onset spinal muscular atrophy to chromosome 5q 11.2–13.3. Nature 344:540, 1990. *Evidence that the infantile and childhood types of spinal muscular atrophy are allelic disorders of a single gene.*

Deng H-X, Hentati A, Tainer JA, et al.: Amyotrophic lateral sclerosis and structural defects in Cu, Zn superoxide dismutase. Science 261:1047, 1993. *Mutations of the gene for superoxide dismutase, important for scavenging of toxic free radicals, cause reduced red cell enzyme activity in familial ALS.*

Martin JB: Molecular genetics in neurology. Ann Neurol 34:757, 1993. *Provides an informative table.*

Pringle CE, Hudson AJ, Munoz DG, et al.: Primary lateral sclerosis: Clinical features, neuropathology, and diagnostic criteria. Brain 115:495, 1992. *The most comprehensive article about an uncommon disease, carefully reported.*

Williams AC (ed.): Motor Neuron Disease. London, Chapman and Hall, 1994. 776 pp. *In addition to offering full clinical accounts of motor neuron disorders, this book reviews current research on pathogenesis of ALS and gives helpful advice about medical management.*

416 SYRINGOMYELIA

Syringomyelia is a disorder of the spinal cord and, often, the lower brain stem, characterized by slowly progressive enlargement of a fluid-filled cyst (syrinx) within the cord or medulla. Most cases are congenital in origin, related to maldevelopment of the cervicomedullary junction; others are caused by arachnoiditis, intraspinal tumor, or trauma.

PATHOLOGY. In congenital cases, the cyst is thought to represent an enormously dilated remnant of the fetal central canal, which usually does not communicate with the fourth ventricle. It is lined by glial tissue, and in places by remnants of ependyma, and contains clear fluid identical to cerebrospinal fluid. Extending from the high cervical level or medulla to the thoracic or lumbar cord, the cavities vary in shape and size at different levels. Most patients have a Chiari type of congenital cerebellar malformation, in which flattened ectopic tonsils descend caudally so as to obstruct both the exit foramina of the fourth ventricle and the subarachnoid space at the foramen magnum.

Acquired syringomyelia may result from basal arachnoiditis, obstructing the cerebrospinal fluid pathways around the foramen magnum, or may develop in a segment of the cord rendered abnormal by an intramedullary tumor, spinal arachnoiditis, or severe traumatic injury. In nontumor cases the cavity is lined only by glia, whereas in tumor cases the cyst wall may contain both tumor and glial cells.

PATHOGENESIS. The mechanism of cyst formation and expansion is poorly understood. In congenital syringomyelia the cavity probably originates before birth as a dilatation of the primitive central canal. Enlargement of the cyst is somehow related to obstruction of the subarachnoid space at the cervicomedullary junction by the ectopic cerebellar tonsils, causing a pressure gradient between the cyst and the subarachnoid space, especially during straining, coughing, or sneezing. The mechanism may be similar in cases of basal arachnoiditis. In patients with spinal arachnoiditis, the cyst may originate in an area of ischemic myelomalacia, and in cases associated with tumor or severe injury there is cystic degeneration of the spinal cord before the syrinx starts to expand. Why the cyst continues to enlarge in these noncommunicating cases is hard to understand, because there is no apparent pressure gradient between the cyst and the subarachnoid space. Obstruction of cerebrospinal fluid circulation due to spinal arachnoiditis may be an important factor.

CLINICAL MANIFESTATIONS. The classic clinical picture of congenital syringomyelia is of a slowly progressive, asymmetric, destructive process in the central portion of the cervical and thoracic spinal cord, damaging the anterior horn cells, the crossing spinothalamic tract fibers, and the lateral corticospinal tracts. This causes muscle weakness and wasting in the hands and arms; scoliosis owing to denervation of paraspinal muscles; loss of arm reflexes; spastic weakness of the lower extremities; and a *dissociated sensory loss* with impaired perception of pain and temperature in the neck, arms, and upper trunk and preserved light touch perception and proprioception. Some patients experience a deep, aching pain in the neck or arms. Symptoms usually begin between 25 and 40 years of age and advance relentlessly for decades, although one third of the patients have long periods of stability. The deficits may worsen suddenly after a fall or after coughing or sneezing. Ten percent of patients develop a painless arthropathy of the shoulder, elbow, or hand. Extension into the medulla may cause nystagmus, dysphagia, or wasting of the tongue, and some patients have hydrocephalus or cerebellar signs related to an associated Chiari malformation.

FIGURE 416–1. Magnetic resonance image of upper spine and foramen magnum in a patient with syringomyelia and a small Chiari I malformation *(single arrow).* The syrinx appears as a dark central area in the cervical and thoracic spinal cord *(double arrows).*

The manifestations of acquired syringomyelia depend on the segment of the spinal cord affected. Posttraumatic syringomyelia, developing in paraplegic or quadriplegic patients months or years after the injury, is revealed by weakness and sensory impairment rising craniad from the transected level. The cases associated with arachnoiditis following previous purulent meningitis, subarachnoid hemorrhage, surgery, trauma, or spinal anesthesia tend to involve the thoracic and lower cervical segments. Syringes associated with intramedullary spinal cord tumor extend for variable distances rostrad or caudad to the tumor.

DIAGNOSIS. Magnetic resonance (MR) imaging, outlines the size and extent of the cavity as well as the presence of cerebellar ectopia, arachnoiditis, or an intraspinal tumor (Fig. 416–1). Electromyography reveals active and chronic denervation in wasted upper extremity muscles, but sensory nerve conduction is normal in the analgesic hand because the lesion is located proximal to the dorsal root ganglia. The cerebrospinal fluid is normal except for a raised protein content in cases associated with tumor or arachnoiditis.

TREATMENT. Various surgical procedures have been devised in the hope of arresting the neurologic deterioration. None has been reliably successful. If hydrocephalus is present, placement of a ventriculoperitoneal shunt may be sufficient to cause the syrinx to collapse, but seldom halts its progression. For congenital syringomyelia associated with cerebellar ectopia, it is customary to perform a posterior decompression of the foramen magnum, ensuring that the fourth ventricle communicates with the subarachnoid space. Acquired syringomyelia is usually treated by decompressing the cyst via a syringoarachnoid, syringopleural, or syringoperitoneal shunt. It has not been established that the outcome of any of these procedures is superior to the natural history of the disease. Some neurosurgical reports suggest that these operations often reduce chronic pain and arrest the progression of neurologic symptoms but long-term follow-up is lacking.

Anderson NE, Willoughby EW, Wrightson P: The natural history and the influence of surgical treatment in syringomyelia. Acta Neurol Scand 71:472, 1985. *A thoughtful critique of the uncertain role of surgical treatment for syringomyelia.*

Oldfield EH, Muraszko K, Shawker TH, Patronas NJ: Pathophysiology of syringomyelia associated with Chiari I malformation of the cerebellar tonsils. Implications for diagnosis and treatment. J Neurosurg 80:3, 1994. *Uses light-tech imaging to analyze fluid dynamics in the syrinx and advocates a new approach to treatment.*

417 THE PHAKOMATOSES

The phakomatoses, or neurocutaneous syndromes, are congenital disorders characterized by disordered growth of ectodermal tissues, producing distinctive skin lesions and malformations or tumors of the nervous system. More than 20 syndromes have been described, the most important of which are neurofibromatosis 1 and 2, tuberous sclerosis, and Sturge-Weber disease.

NEUROFIBROMATOSIS 1 (von Recklinghausen's Disease)

Neurofibromatosis 1 is characterized by multiple café au lait spots on the skin, multiple peripheral nerve tumors, and a variety of other dysplastic abnormalities of the skin, nervous system, bones, endocrine organs, and blood vessels. It is one of the most common genetic diseases, occurring approximately once in every 3000 births. It is inherited as an autosomal dominant trait, but 40 to 60% of cases are clinically sporadic. Even allowing for the difficulty of detecting the trait in mild cases, there seems to be a remarkably high mutation rate, on the order of 10^{-4} per locus per generation. The responsible gene, which occupies a 300-kilobase region of chromosome 17q, codes for a GTPase-activating protein (neurofibromin), which functions as a tumor suppressor.

PATHOLOGY. The peripheral nerve tumors are of two types, schwannomas and neurofibromas, the latter derived from both Schwann cells and perineural fibroblasts. Neurofibromas of sensory nerve twigs produce the distinctive subcutaneous nodules; in peripheral nerve trunks the tumor appears as a fusiform enlargement or plexiform neuroma. Schwannomas arise in cranial and spinal nerve roots and also in peripheral nerve trunks. Both types of tumor occasionally become malignant. The brain may show disordered architecture, hamartomas, gliomas, and meningiomas.

CLINICAL MANIFESTATIONS. Some manifestations are congenital, but most appear during childhood and adult life. Café au lait spots become larger and more numerous with age; most patients eventually have more than six spots greater than 1.5 cm in diameter. Other skin lesions include freckles (axillary freckles being specific to this disease); soft pedunculated cutaneous neurofibromas, and firm subcutaneous neurofibromas.

Plexiform neurofibromas may grow to enormous size, leading to grotesque overgrowth of soft tissues and bone in a limb or around the orbit. Enlarging nerve trunk tumors may cause pain and impair sensory-motor function; intraspinal nerve root tumors do the same and also compress the spinal cord. Gliomas of the optic nerve and chiasm are the most frequent intracranial tumor; they usually behave indolently as hamartomas do. A hamartoma of the hypothalamus may cause precocious puberty.

About 10% of children are mentally deficient, and about 10% develop seizures, half in association with an intracranial tumor. Kyphoscoliosis, dysplasia of the skull, bowed legs, and other bone abnormalities are common. Pheochromocytoma occurs in about 5% of patients, usually in adult life. Hypertension may result from renal artery dysplasia.

DIAGNOSIS. The diagnosis is usually clinically evident, but biopsy of a neurofibroma can be diagnostic in cryptic cases. Spinal nerve root tumors often have a dumbbell shape, with intraspinal and extraspinal components; these are most readily identified on MRI. For diagnosis of intracranial tumors and hamartomas either CT or MRI is suitable.

TREATMENT. Most patients live a normal life with few or no symptoms. Small cutaneous or subcutaneous neurofibromas can be removed if they are painful or frequently irritated, but large plexiform neurofibromas usually should be left alone. A few become malignant with continued invasion and fatal outcome. Symptomatic peripheral nerve trunk schwannomas can sometimes be removed safely by an experienced surgeon. Intraspinal and intracranial schwannomas are approached in the usual surgical fashion. Optic nerve gliomas are generally treated with radiation, but it is not clear

NEUROFIBROMATOSIS 2

This rare disease is characterized by the occurrence of bilateral acoustic neuromas and often other intracranial tumors, such as meningiomas and ependymomas. A few café au lait spots are present in 42% of cases. The disease is inherited as an autosomal dominant trait, but 50% of cases are new mutations. The responsible gene, located on chromosome 22q, codes for a cytoskeletal protein (merlin) which is presumed to function as a tumor suppressor. Family members at risk for the disease should be screened regularly with hearing tests and brain stem auditory evoked responses.

TUBEROUS SCLEROSIS

The phenotype of tuberous sclerosis consists of mental deficiency, epilepsy, and a characteristic facial eruption known as adenoma sebaceum. The disease is inherited as an autosomal dominant trait, but about 80% of the cases are sporadic, owing to new mutations. Two separate gene loci have been linked to the syndrome, one on chromosome 9q and the other on chromosome 16p.

PATHOLOGY. The facial papules of adenoma sebaceum are angiofibromas. The cerebral hemispheres contain multiple hamartomas characterized by disordered architecture, proliferating and abnormal astrocytes, and deposits of calcium. The common retinal hamartomas are also probably of glial origin. Visceral lesions include multiple rhabdomyomas of the heart, multiple angiomyolipomas of the kidneys, and cystic transformation of the lungs by proliferating fibrous, muscular, and vascular tissue.

CLINICAL MANIFESTATIONS. Mental deficiency may be mild or severe, but one third of affected individuals have normal or even superior intelligence. Seizures occur in 80% of cases, usually starting before the age of 5, and are often difficult to control with medication. In infants the seizures often take the form of infantile spasms; these children tend to be more severely impaired mentally. Occasionally, diagnosis escapes attention until late adolescence or adult life, when investigation of a seizure disorder of new onset discloses subtle skin lesions or multiple retinal or intracranial hamartomas.

Nearly all patients have distinctive skin lesions. Hypopigmented spots are present from the time of birth in nearly 100% of patients; they are more numerous on the trunk and are easier to see with a Wood's lamp. The next most common is adenoma sebaceum, a papular, salmon-colored eruption about the center of the face and in the nasolabial folds. It usually becomes more prominent after puberty. Leathery "shagreen" patches over the lower back and fibromas of the nailbeds affect perhaps 40% of patients.

Retinal hamartomas affect about half the patients. About 30% of patients have cardiac rhabdomyomas, which sometimes cause arrhythmia or congestive heart failure. Renal tumors occur in two thirds of patients and are usually asymptomatic, although pain and bleeding can occur. Cystic disease of the lungs, an uncommon complication, mainly affects women over the age of 20; the symptoms include pneumothorax, dyspnea, cyanosis, and cor pulmonale.

DIAGNOSIS. Clinical diagnosis often is obvious. MRI is the procedure of choice and identifies both calcified and uncalcified tubers and nodules. Adenoma sebaceum, ungual fibromas, and hypopigmented spots are diagnostically specific, but retinal hamartomas also occur in neurofibromatosis.

Treatment is confined to symptomatic control of the epilepsy and to surgical therapy of the occasional hamartoma that undergoes gliomatous changes and enlarges to produce symptoms.

STURGE-WEBER SYNDROME

The Sturge-Weber syndrome is a nonhereditary, congenital disorder of facial and cerebral blood vessels characterized by a facial angioma (port-wine stain), seizures, and mental deficiency. The condition involves a defect of embryonic development, with persistence of a vascular plexus in the cephalic portion of the neural tube. The incidence is about 5 in 100,000 births.

The facial angioma is usually unilateral but may extend to the other side and conforms largely but not strictly to trigeminal nerve subdivisions. There may be cavernous angiomas of the tongue, gums, or mouth, and choroidal angiomas may cause congenital glaucoma. An angioma of the occipital and parietal leptomeninges accompanies the facial nevus on the same side, and the underlying cerebral hemisphere is atrophic, with degenerative changes and de-

cortex. The cortical calcifications and atrophy are easily seen on brain CT. Neurologic symptoms develop in infancy or early childhood, consisting of focal or generalized seizures. Half of the children become mentally impaired, and one third develop a hemiparesis. When seizures are difficult to control with medication, early surgical removal of the affected part of the brain may improve control and prevent intellectual deterioration.

Gomez MR (ed.): Tuberous Sclerosis, 2nd ed. New York, Raven Press, 1988. *Gives detailed information about multiple organ involvement in tuberous sclerosis, with excellent illustrations of the skin and retinal lesions.*

Gutmann DH, Wood DL, Collins FS: Identification of the neurofibromatosis type 1 gene product. Proc Natl Acad Sci 88:9658, 1991. *The title identifies the content.*

Mulvihill JJ (moderator): Neurofibromatosis 1 (Recklinghausen disease) and neurofibromatosis 2 (bilateral acoustic neurofibromatosis): An update. Ann Intern Med 113:39, 1990. *A succinct review of clinical and genetic features of both syndromes.*

Trofatter JA, MacCollin MM, Rutter JL, et al.: A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. Cell 72:791, 1993. *Characterizes the probable structure of the causative gene abnormality.*

Section Six—Cerebrovascular Diseases

William A. Pulsinelli

418 CEREBROVASCULAR DISEASES—PRINCIPLES

The family of cerebrovascular diseases can be classified according to whether they affect the brain's vascular supply either focally or diffusely (Fig. 418–1). The generic term "stroke" signifies the abrupt impairment of brain function caused by a variety of pathologic changes involving one (focal) or several (multifocal) intracranial or extracranial blood vessels. Approximately 80% of all strokes are caused by too little blood flow (ischemic stroke), and the remaining 20% are nearly equally divided between hemorrhage into brain tissue (parenchymatous hemorrhage) or the surrounding subarachnoid space (subarachnoid hemorrhage). In contrast, diseases that affect the heart or the systemic circulation cause generalized hypoperfusion and diffuse brain dysfunction or injury. Ischemic stroke and the hypoperfusion syndromes affecting the brain share much pathophysiology, and both processes are considered together in Ch. 419; hemorrhagic stroke is addressed in Ch. 420.

EPIDEMIOLOGY

The annual incidence and death rate for stroke have declined steadily in the United States throughout the twentieth century and for most European countries and Japan since approximately 1960. In the United States, a 1% per year decrease in the annual mortality rate from stroke recorded since 1915 accelerated in the early 1970's to approximately 5% per year. A recent analysis indicates that the stroke incidence has stabilized at approximately 0.5 to 1.0 per 1000 population. Incidence rates in western European countries are slightly higher (1.5 per 1000), but several eastern European countries and Japan have rates of 3 per 1000 based at least partly on environmental, dietary, and smoking habits. At these current rates, stroke remains the third leading cause of medically related deaths and the second most frequent cause of neurologic morbidity in developed countries.

Several other important facts about stroke incidence have emerged: incidence and death rate for stroke are higher among blacks than whites in the United States; approximately similar rates affect men and women, in contrast to the male predominance for myocardial infarction; and there is a strikingly higher incidence (20 to 30 per 1000) for those over age 75.

CEREBROVASCULAR ANATOMY

Since most strokes are caused by abnormalities within the cerebral circulation, an understanding of cerebrovascular anatomy helps in arriving at the correct diagnosis and determining the underlying pathogenesis and prognosis.

The brain is supplied by four major arteries: the left and right internal carotid and vertebral arteries (Fig. 418–2). The left common carotid artery arises from the aortic arch, but the other vessels originate from branches of the aorta; the right common carotid artery stems from the innominate artery, and the left and right vertebral arteries take off from their respective subclavian arteries.

INTERNAL CAROTID ARTERIES. Each common carotid artery bifurcates into an internal and external carotid artery in most

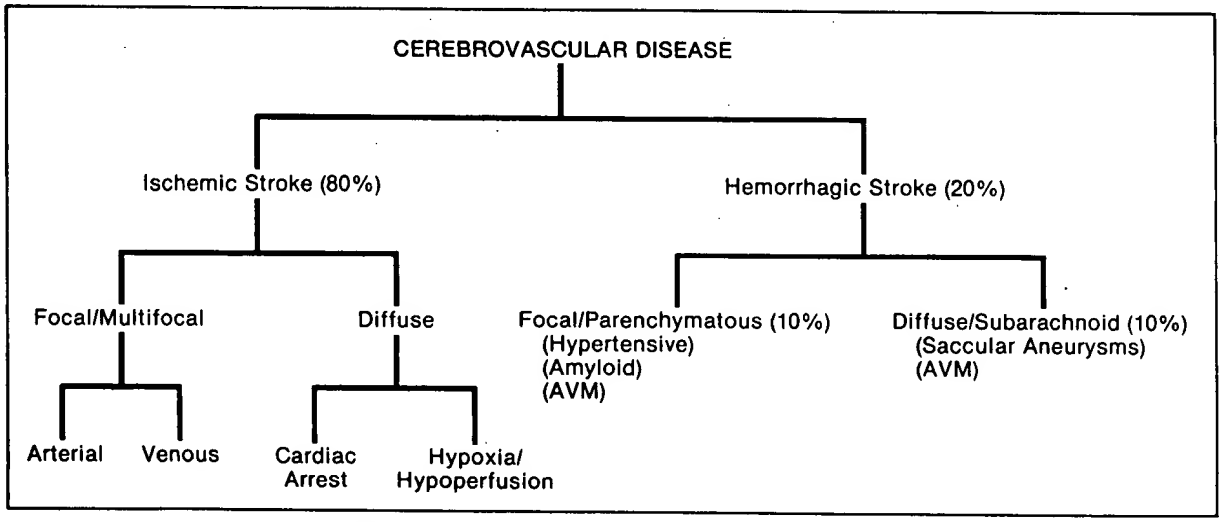


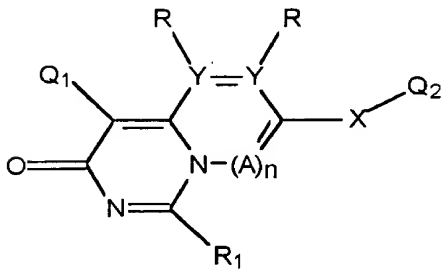
FIGURE 418–1. Classification of cerebrovascular disease.

useful in treating various conditions associated with p38 activation.

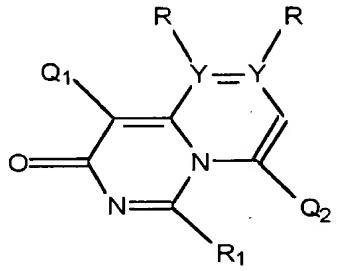
SUMMARY OF THE INVENTION

The present invention solves this problem by providing compounds which demonstrate strong and specific inhibition of p38.

These compounds have the general formula:



(Ia) or



(Ib),

wherein each of Q_1 and Q_2 are independently selected from 5-6 membered aromatic carbocyclic or heterocyclic ring systems, or 8-10 membered bicyclic ring systems comprising aromatic carbocyclic rings, aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring.

The rings that make up Q_1 are substituted with 1 to 4 substituents, each of which is independently selected from halo; C_1-C_3 alkyl optionally substituted with NR'_2, OR', CO_2R' or $CONR'_2$; O-(C_1-C_3)-alkyl optionally substituted with NR'_2, OR', CO_2R' or $CONR'_2$; NR'_2; OCF_3; CF_3; NO_2; CO_2R'; [CONR'] CONHR'; SR'; $S(O_2)N(R')_2$; SCF_3; CN; $N(R')C(O)R^4$; $N(R')C(O)OR^4$; $N(R')C(O)C(O)R^4$; $N(R')S(O_2)R^4$; $N(R')R^4$; $N(R^4)_2$; OR^4; $OC(O)R^4$; $OP(O)_3H_2$; or [N=C-$N(R')_2$] N=CH-$N(R')_2$.

The rings that make up Q_2 are optionally substituted with up to 4 substituents, each of which is independently selected from halo; C_1-C_3 straight or

branched alkyl optionally substituted with NR'_2, OR', CO_2R', $S(O_2)N(R')_2$, [N=C-N(R')$_2$] N=CH-N(R')$_2$, R^3, or $CONR'_2$; O-(C_1-C_3)-alkyl; O-(C_1-C_3)-alkyl optionally substituted with NR'_2, OR', CO_2R', $S(O_2)N(R')_2$, [N=C-N(R')$_2$] N=CH-N(R')$_2$, R^3, or $CONR'_2$; NR'_2; OCF_3; CF_3; NO_2; CO_2R'; [CONR'] CONHR'; R^3; OR^3; [NR^3] NHR^3; SR^3; $C(O)R^3$; $C(O)N(R')R^3$; $C(O)OR^3$; SR'; $S(O_2)N(R')_2$; SCF_3; [N=C-N(R')$_2$] N=CH-N(R')$_2$, or CN.

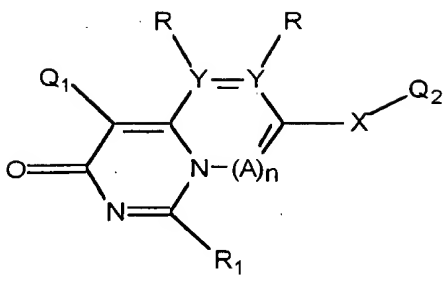
R' is selected from hydrogen, (C_1-C_3)-alkyl; (C_2-C_3)-alkenyl or alkynyl; phenyl or phenyl substituted with 1 to 3 substituents independently selected from halo, methoxy, cyano, nitro, amino, hydroxy, methyl or ethyl.

R^3 is selected from 5-6 membered aromatic carbocyclic or heterocyclic ring systems.

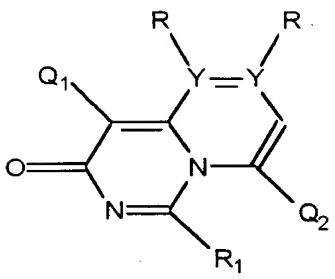
R^4 is (C_1-C_4)-alkyl optionally substituted with $N(R')_2$, OR', CO_2R', $CON(R')_2$, or $SO_2N(R^2)_2$; or a 5-6 membered carbocyclic or heterocyclic ring system optionally substituted with $N(R')_2$, OR', CO_2R', $CON(R')_2$, or $SO_2N(R^2)_2$.

X is selected from -S-, -O-, $-S(O_2)-$, -S(O)-, $-S(O_2)-N(R^2)-$, $-N(R^2)-S(O_2)-$, $-N(R^2)-C(O)O-$, $-O-C(O)-N(R^2)$, -C(O)-, -C(O)O-, -O-C(O)-, $-C(O)-N(R^2)-$, $-N(R^2)-C(O)-$, $-N(R^2)-$, $-C(R^2)_2-$, or $-C(OR^2)_2-$.

Each R is independently selected from hydrogen, $-R^2$, $-N(R^2)_2$, $-OR^2$, SR^2, $-C(O)-N(R^2)_2$, $-S(O_2)-N(R^2)_2$, or $-C(O)-OR^2$, wherein two adjacent R are optionally bound to one another and, together with each Y to which they are respectively bound, form a 4-8 membered carbocyclic or heterocyclic ring;



(Ia) or



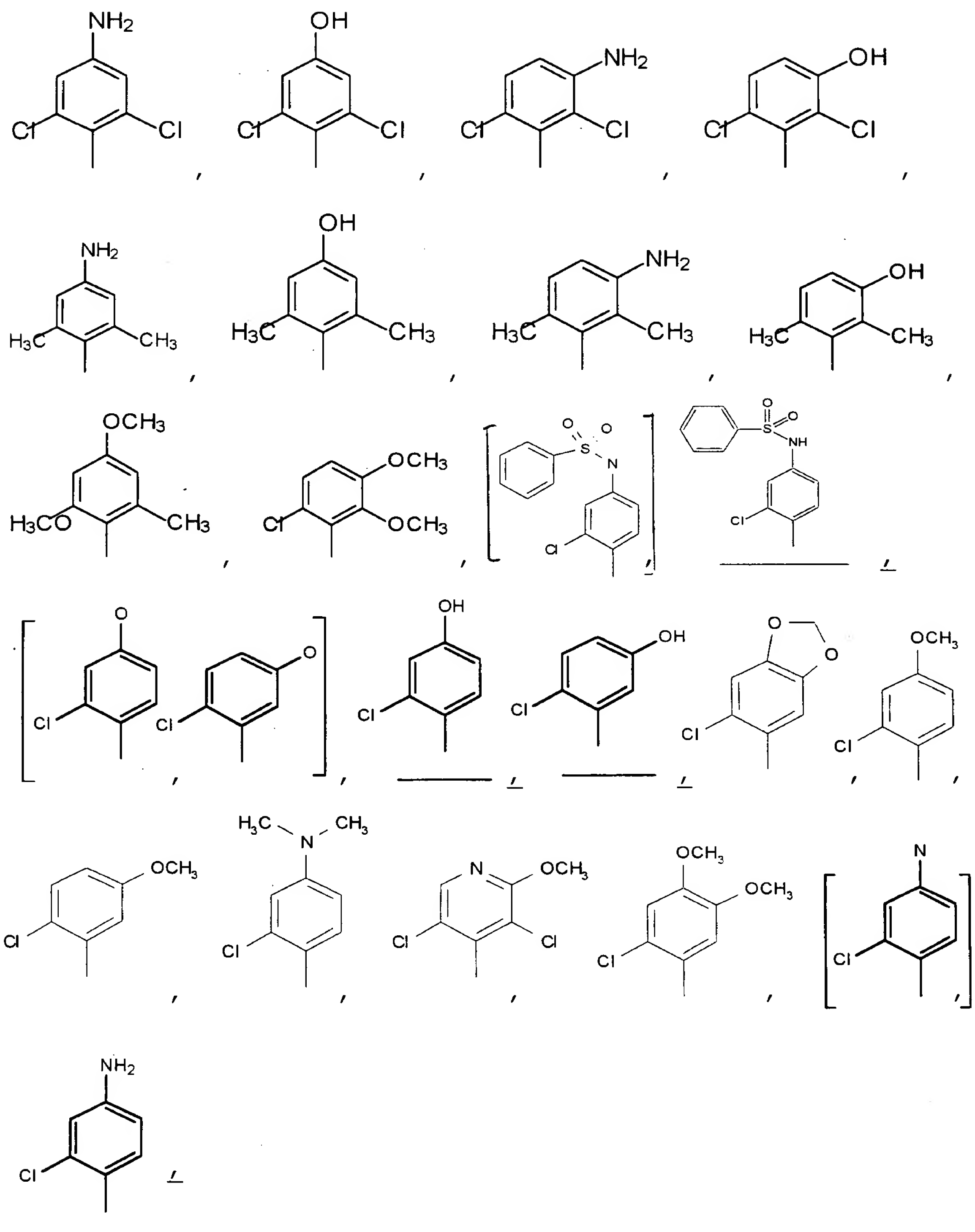
(Ib),

wherein each of Q_1 and Q_2 are independently selected from 5-6 membered aromatic carbocyclic or heterocyclic ring systems, or 8-10 membered bicyclic ring systems comprising aromatic carbocyclic rings, aromatic heterocyclic rings or a combination of an aromatic carbocyclic ring and an aromatic heterocyclic ring.

The rings that make up Q_1 are substituted with 1 to 4 substituents, each of which is independently selected from halo; C_1-C_3 alkyl optionally substituted with NR'_2, OR', CO_2R' or $CONR'_2$; O-(C_1-C_3)-alkyl optionally substituted with NR'_2, OR', CO_2R' or $CONR'_2$; NR'_2; OCF_3; CF_3; NO_2; CO_2R'; [CONR'] CONHR'; SR'; $S(O_2)N(R')_2$; SCF_3; CN; $N(R')C(O)R^4$; $N(R')C(O)OR^4$; $N(R')C(O)C(O)R^4$; $N(R')S(O_2)R^4$; $N(R')R^4$; $N(R^4)_2$; OR^4; $OC(O)R^4$; $OP(O)_3H_2$; or [N=C-N(R')$_2$] N=CH-N(R')$_2$.

The rings that make up Q_2 are optionally substituted with up to 4 substituents, each of which is independently selected from halo; C_1-C_3 straight or branched alkyl optionally substituted with NR'_2, OR', CO_2R', $S(O_2)N(R')_2$, [N=C-N(R')$_2$] N=CH-N(R')$_2$, R^3, or $CONR'_2$; O-(C_1-C_3)-alkyl; O-(C_1-C_3)-alkyl optionally substituted with NR'_2, OR', CO_2R', $S(O_2)N(R')_2$, [N=C-N(R')$_2$] N=CH-N(R')$_2$, R^3, or $CONR'_2$; NR'_2; OCF_3; CF_3; NO_2; CO_2R'; [CONR'] CONHR'; R^3; OR^3; [NR^3] NHR^3; SR^3; $C(O)R^3$;

$C(O)N(R')R^3$; $C(O)OR^3$; SR'; $S(O_2)N(R')_2$; SCF_3; $[N=C-N(R')_2]$ $\underline{N=CH-N(R')_2}$, or CN.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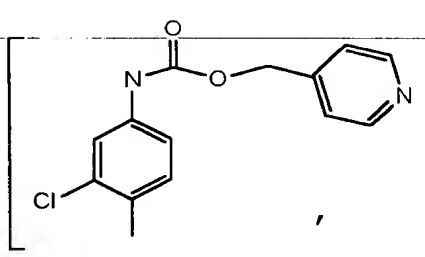
NH2 Cl ,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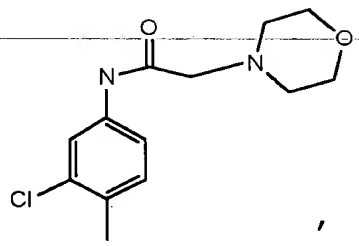
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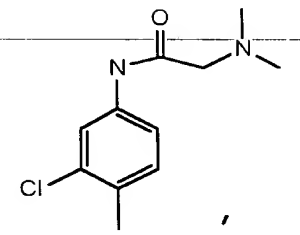
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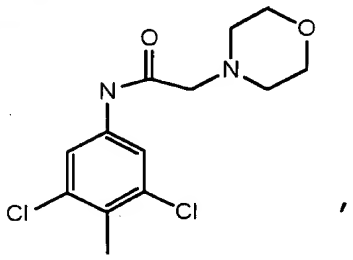
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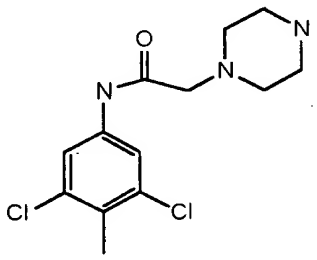
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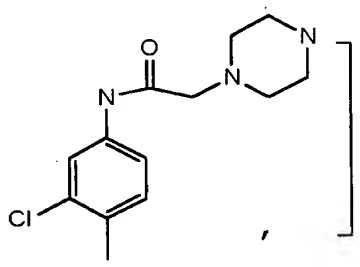
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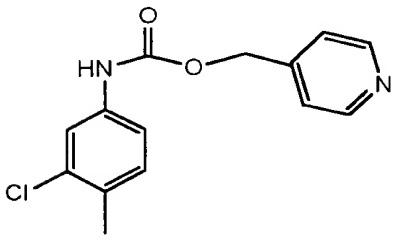
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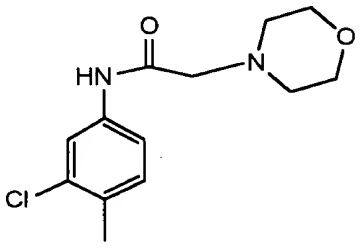
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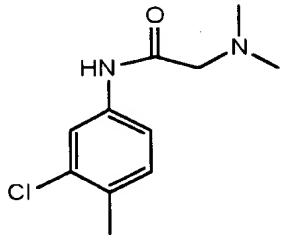
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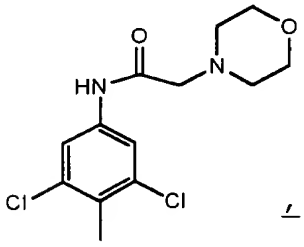
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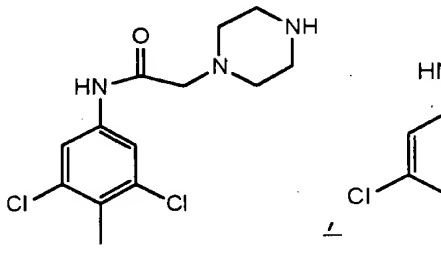
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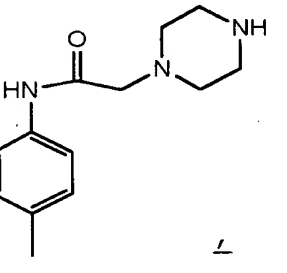
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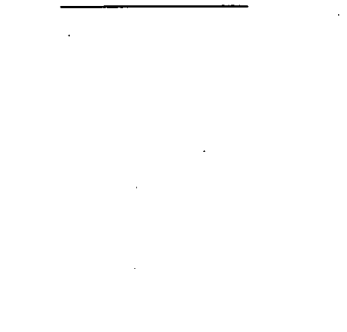
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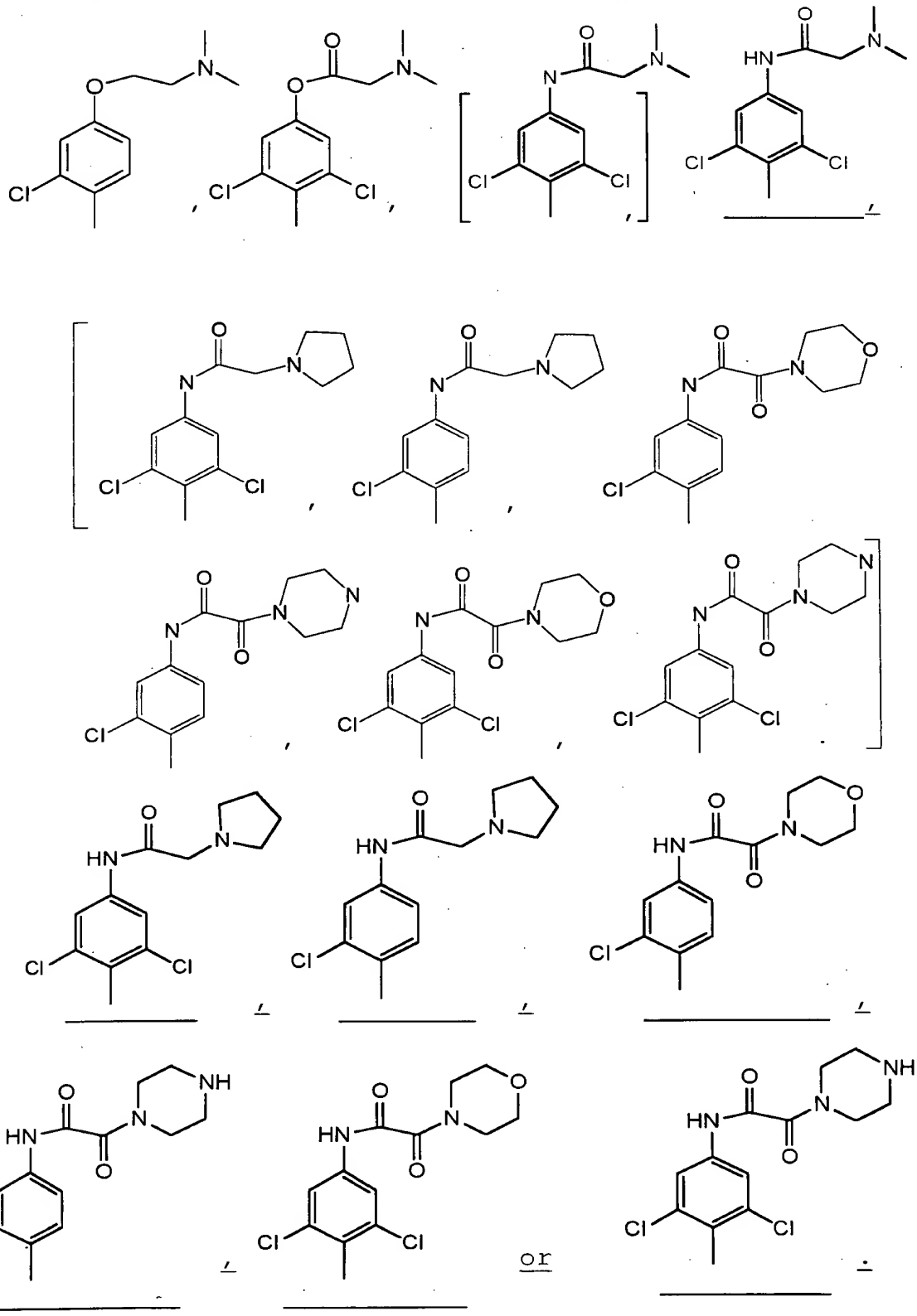

Table 4. Formula Ig Inhibitors

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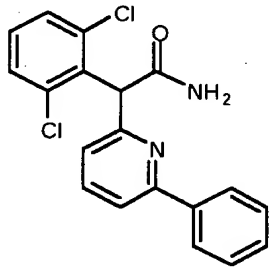
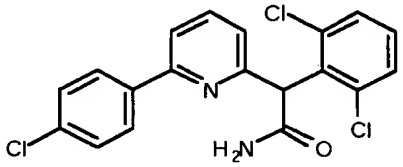
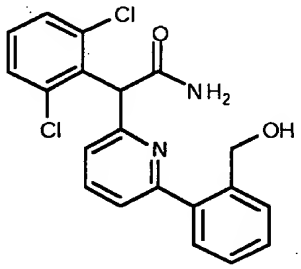
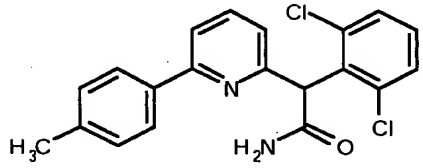
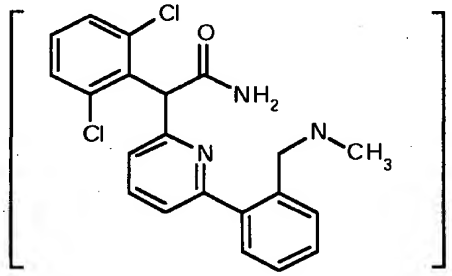
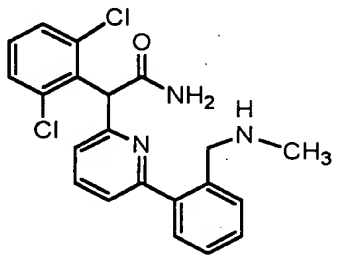
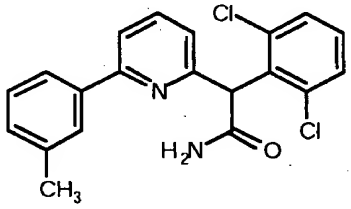
202/ 301		310	
302		311	
303	 	312	

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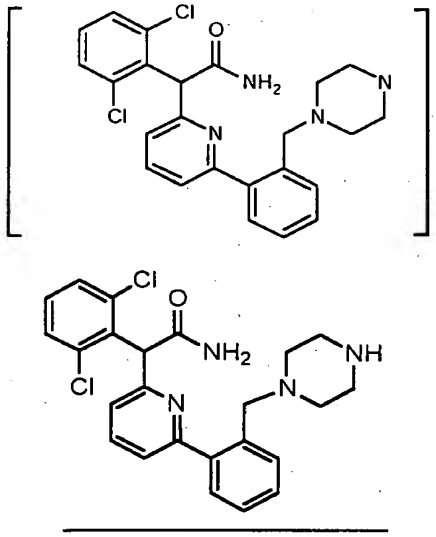
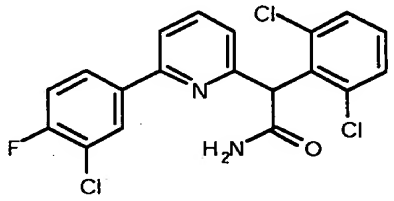
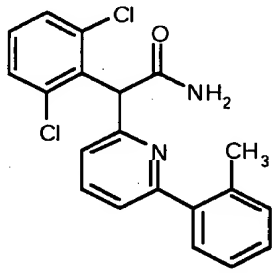
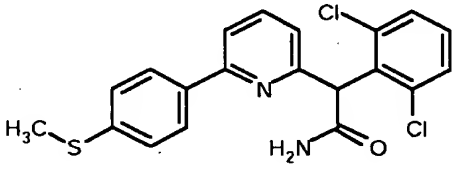
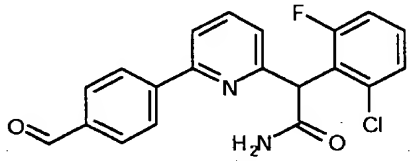
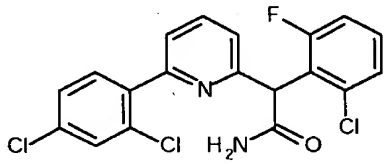
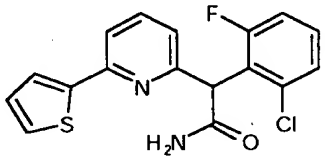
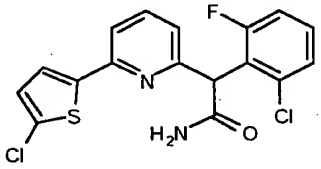
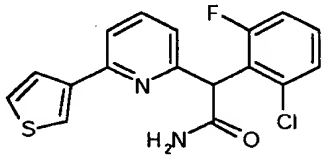
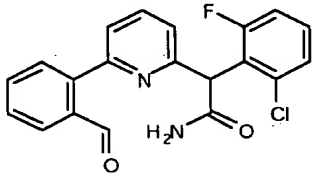
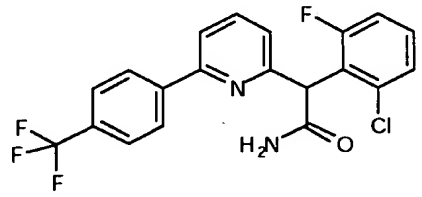
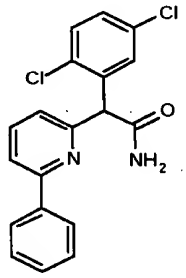
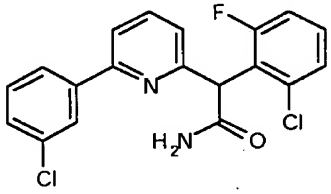
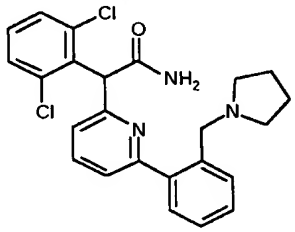
304		313	
305		314	

cpd #	structure	cpd #	structure
337		346	
338		347	
339		348	
340		349	
341		350	

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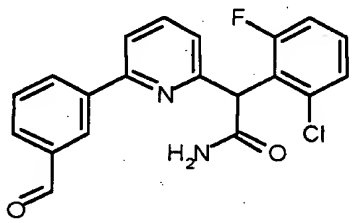
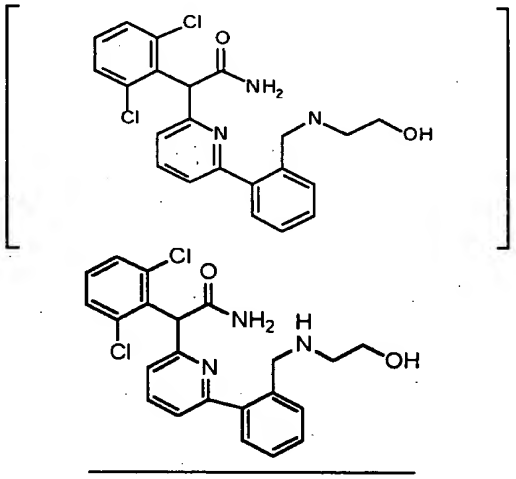
342		351	

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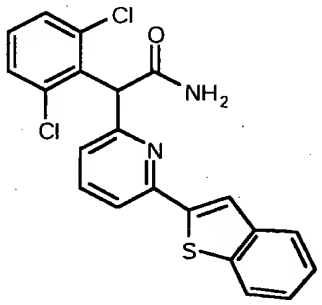
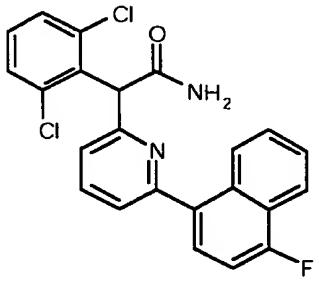
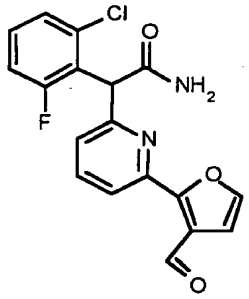
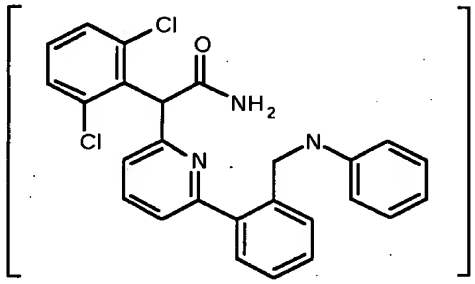
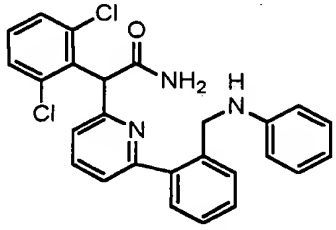
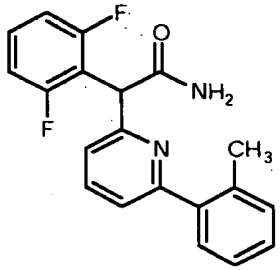
356		365	
357		366	
358	 	367	

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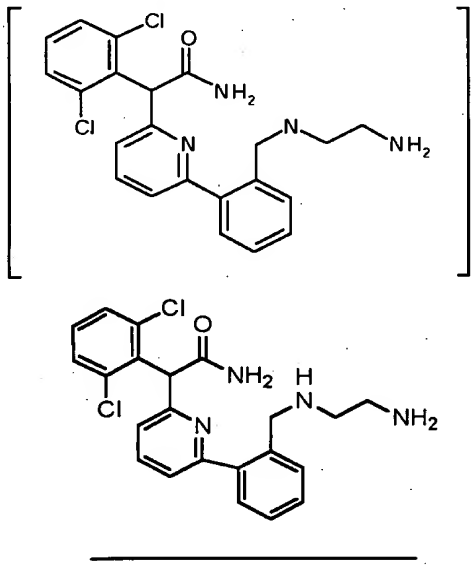
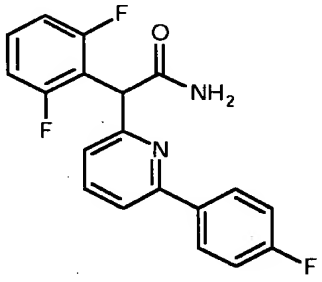
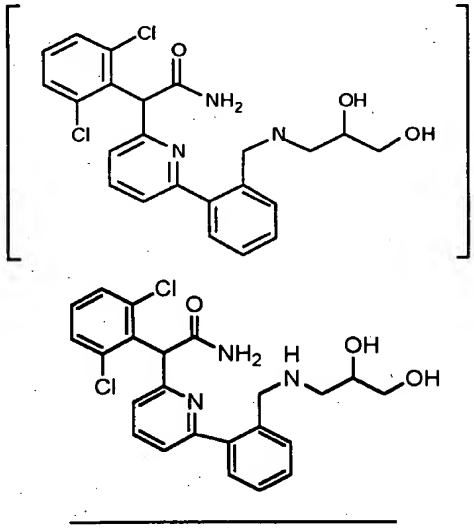
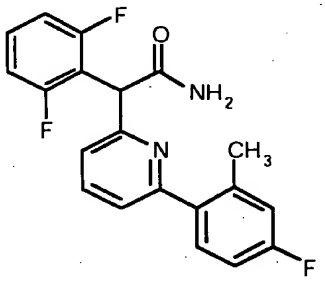
359		368	
360		369	

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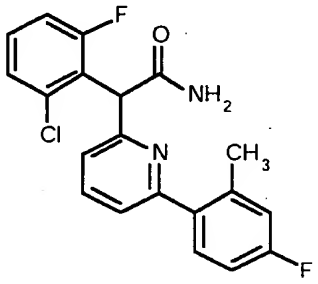
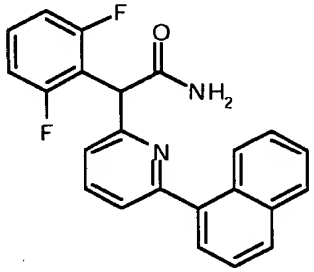
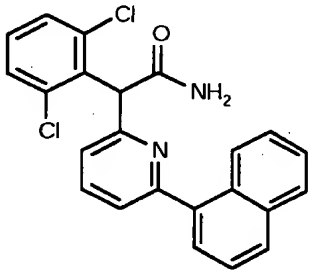
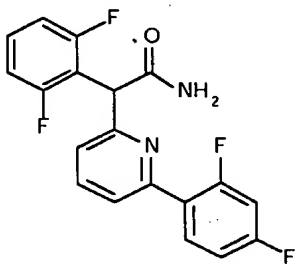
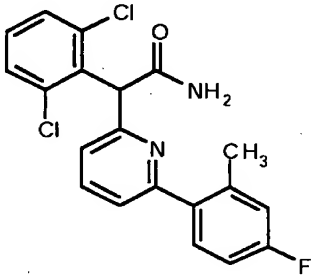
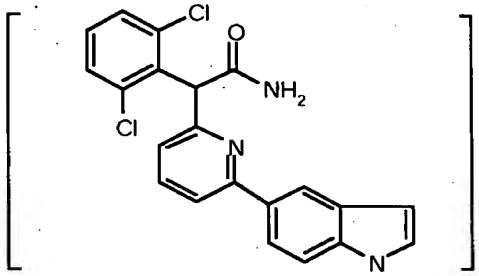
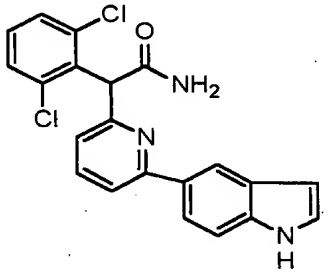
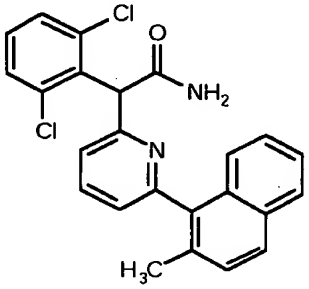
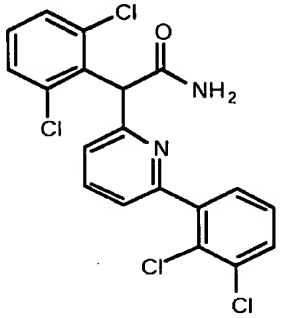
361		370	
362		371	
363		372	 
373		382	

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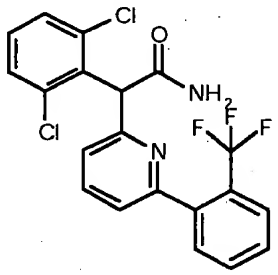
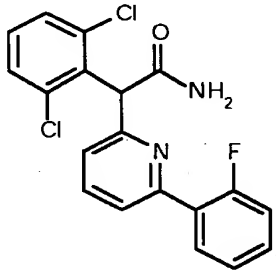
374		383	

cmpd #	kinase IC50	cell IL-1 IC50	cell TNF IC50	WB IL-1 IC50	WB TNF IC50	WB IL-6 IC50
409	+++	+++	+++	+	+	++
410	+++	+++	+++	++	++	++
411	+++	+++	+++	+	+	+
412	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

For kinase IC50 values, "+++" represents [>] $\leq$0.1 μM, "++" represents between 0.1 and 1.0 μM, and "+" represents [<] $\geq$1.0 μM. For cellular IL-1 and TNF values, "+++" represents [>] $\leq$0.1 μM, "++" represents between 0.1 and 0.5 μM, and "+" represents [<] $\geq$0.5 μM. For all whole blood ("WB") assay values, "+++" represents [>] $\leq$0.25 μM, "++" represents between 0.25 and 0.5 μM, and "+" represents [<] $\geq$0.5 μM. In all assays indicated in the table above, "N.D." represents value not determined.

Other p38 inhibitors of this invention will also inhibit phosphorylation of EGF receptor peptide, and the production of IL-1, TNF and IL-6, as well as IL-8 in LPS-stimulated PBMCs or in whole blood.

D. Inhibition of IL-6 and IL-8 Production in IL-1-Stimulated PBMCs

This assay was carried out on PBMCs exactly the same as above except that 50 μl of an IL-1b working stock solution (2 ng/ml in cell culture medium) was added to the assay instead of the (LPS) working stock solution.

Cell culture supernatants were harvested as described above and analyzed by ELISA for levels of IL-6 Endogen, #EH2-IL6) and IL-8 (Endogen, #EH2-IL8) according to the instructions of the manufacturer. The